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ANALYSIS OF DRUGS AND DRUG METABOLITES IN BODY FLUIDS BY CVA-MASS SPECTROMETRY

by

Seth R. Abbott
Kay O. Loeffler

Varian Associates
Palo Alto, California 94303

and Supplementary Report

ANALYSIS OF MORPHINE IN URINE BY CVA-MASS SPECTROMETRY

by

Seth R. Abbott
James T. Arnold (and others)
Kay O. Loeffler

Varian Associates
Palo Alto, California 94303

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Final Report

Contract DAAD05-72-C-0111

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U. S. ARMY LAND WARFARE LABORATORY
Aberdeen Proving Ground, Maryland 21005

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mass spectrums of drugs of abuse	amphetamines	alkaloids

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Analysis of Drugs and Drug Metabolites in Body Fluids by CVA-Mass Spectrometry:

The objective of this work as stated in Section F of Contract DAAD05-72-C-0111 was "... to determine if it is feasible to:

a. detect that a person is under the influence of a drug, and
b. determine what this drug is

Continued on Reverse

Block 20. ABSTRACT CON'T

by analysing in real time either a breath sample, an air sample containing materials transpired through the skin or a body fluid sample with the mass spectrometer (CVA) detection instrument."

Tests were conducted at Varian Associates and at San Francisco General Hospital. These revealed that urine, blood (Sect VII), and gastric fluids are suitable media for drug detection. A wide range of drugs (narcotics, barbiturates, phenothiazines, tranquilizers) were rapidly detected in overdose and therapeutic dose samples of these media. Traces of morphine were detected in urines 3-4 days after heroin dosage. Codeine was detected in heroin addict urines at an unexpectedly high level and subsequent studies indicated that codeine level in urine can be used to distinguish the hard-core heroin user from the experimenter or new user.

Due to the low volatility and permeability of most drugs at body temperature, detection was not achieved in samples derived from breath, saliva, or skin wipings.

The selection of drugs was limited by their availability from patients at the hospital. Thus the majority of this work is on measuring the quantities of narcotics, barbiturates, tranquilizers, and their metabolites in urine samples. Additional work on hallucinogens and amphetamines would be desirable, as well as the further development of technique for using blood samples.

This report demonstrates that in its current state of development, CVA-Mass Spectroscopy is suitable for rapid detection of many drugs and metabolites in body fluids at both the therapeutic and overdose level.

Supplementary Report: Analysis of Morphine in Urine by CVA-Mass Spectrometry:

CVA sensitivity to morphine in urine of 10 ng/ml was demonstrated. This sensitivity will allow detection of morphine in unhydrolyzed urines of heroin addicts within 24 hours of dose, and detection of morphine in hydrolyzed urines of heroin addicts greater than 3-4 days after dose. Several derivatization agents were investigated and it was determined that a methylating agent gave the best results for this application. The effect of storage conditions on sample concentration was also investigated.

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FOREWORD

This effort was sponsored by the U. S. Army Land Warfare Laboratory (USALWL), Applied Physics Branch, under the technical supervision of H. Clay McDowell. This project was designated 02-P-72, Metabolized Drug Detection.

The authors would like to thank Dr. Charles E. Becker (Head of Clinical Pharmacology, San Francisco General Hospital) for his collaboration in the drug poisoning analysis studies, and both Dr. Becker and Mr. Udo Boerner (Toxicologist, San Francisco Public Health Service) for their collaboration in the heroin abuse studies.

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ABSTRACT

The objective of this work as stated in Section F of Contract DAAD05-72-C-0111 was "... to determine if it is feasible to:

a. detect that a person is under the influence of a drug, and

b. determine what this drug is

by analysing in real time either a breath sample, an air sample containing materials transpired through the skin or a body fluid sample with the mass spectrometer (CVA) detection instrument."

Tests were conducted at Varian Associates and at San Francisco General Hospital. These revealed that urine, blood (Sect. VII), and gastric fluids are suitable media for drug detection. A wide range of drugs (narcotics, barbiturates, phenothiazines, tranquilizers) were rapidly detected in overdose and therapeutic dose samples of these media. Traces of morphine were detected in urines 3-4 days after heroin dosage. Codeine was detected in heroin addict urines at an unexpectedly high level and subsequent studies indicated that codeine level in urine can be used to distinguish the hard-core heroin user from the experimenter or new user.

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This report demonstrates that in its current state of development, CVA-Mass Spectroscopy is suitable for rapid detection of many drugs and metabolites in body fluids at both the therapeutic and overdose level.

I. The Problem - Drug Abuse

The abuse of drugs and narcotic dependence is a problem for both civilian society and the military. The complexity of the problem involves all areas of public interest and control - law, medicine, law enforcement.

The drug problem victimizes not only the abuser but the society from which he must steal to support his habit, the victims of automobile accidents caused by a "drugged" driver, and the taxpayer who must support hospital care and law enforcement budgets in the drug abuse area. The serviceman under the influence of drugs is a threat to his unit in combat situations.

Since military service is a world-wide operation, exposure to illicit local drugs (i.e. - heroin, hashish) is unavoidable. The drug abuse problem has generated considerable unfavorable publicity for the Army in Southeast Asia.

The military drug problem in Southeast Asia is not entirely new. A statistical study of fatal narcotism in military personnel[1] between 1918-1970 indicated about 70% of such cases occurred in Asia - principally during the Korean War. This is being repeated in Viet Nam.

In civilian life, a general feeling of discontent combined with medical irresponsibility in drug prescription and the panacea approach of the pharmaceutical companies has led to a problem in the affluent class which previously was localized in the lower socioeconomic class.

A recent study of routine drinking driver investigations in Santa Clara County, CA indicated concurrent drug use in 21% of the cases.[2] The barbiturates, glutethimide and meprobamate were commonly found.

The drug abuse situation presents an increased workload to civilian, forensic and military analytical laboratories. The goals of analytical development in this area are reduced time and cost of analysis, automation, and concomitant reduction in training of operators.

[1] R. C. Froede and C. J. Stahl, J. Forensic Sci., 16, 199 (1971)

[2] B. Finkle et al., J. Forensic Sci. 13, 236 (1968).

In the case of LSD and marijuana detection (important in driver testing) an additional goal of increased sensitivity over current methods exists.

The development of CVA-Mass Spectrometry addresses itself to these goals.

II. The Concept of CVA-Mass Spectrometry

Mass spectrometry consists of ionizing and fragmenting a compound and recording its fragmentation pattern according to mass.

In CVA-Mass Spectrometry a biological fluid extract is injected (syringe) into a 1/8" x 5" heatable, silanized stainless steel inlet tube. The inlet temperature is chosen so as to vaporize the sample constituent(s) of interest. A sample pump draws the vapor into a three-stage, dimethylsilicone membrane molecular separator, with inter-stage pumping.

At a selected membrane temperature, the organic drug components are absorbed or dissolved in the membrane. The rate of transfer, Q, of a diffusing component across the membrane is a function of the solubility and diffusion coefficient of the component in the membrane.

$$Q = P\,A\,\frac{(p_1 - p_2)}{l}$$

Q = rate of transfer of diffusing component across membrane, in torr-liters/sec.
$p_1 - p_2$ = partial pressure differential of component across membrane.
l = membrane thickness.

$$P = D\,S$$

P = permeability coefficient.
A = membrane area.
D = diffusion coefficient of component across membrane.
S = solubility of component in membrane.

The permanent air gases, macromolecules and highly polar compounds (i.e.,sugars) have low membrane permeability relative to most organic drugs and metabolites. At the high membrane temperature used to analyze drugs, volatile organic solvents used in biological fluid extracts (i.e., - benzene, chloroform, ether) have relatively low permeability.

Thus, the membrane separator preferentially removes organic drug components from the sample vapor, passing them into the low pressure system of a quadrupole mass analyzer, where the components are ionized with sufficient energy (60 eV electron bombardment) to cause fragmentation of the molecules. The resulting positively-charged ions are accelerated into the quadrupole electric field which disperses and permits relative abundance measurements of ions of a given mass to charge ratio. The record of ion abundance versus mass, the fragmentation pattern, is used to identify the drugs present.

Previously, mixtures to be studied in mass spectrometry required prior gas chromatographic (GC) separation into pure components, with the GC effluent admitted into the mass analyzer through a molecular separator interface (GC-MS). The use of GC presented problems in the drug analysis area:

(1) lack of a universal column and column temperature system for drugs,
(2) requirement of derivatization for drugs with poor chromatographic properties (i.e. adsorption on column, thermal degradation on column)
(3) the GC scan of 15 minutes - 1 hour as the time-limiting step in the system is not amenable to high-volume screening.
(4) maintenance of two instruments (GC and MS).

The key concept of CVA-Mass Spectrometry is that the non-drug biological fluid extract components that are membrane-permeable, do not produce abundant ions above m/e 150. This is illustrated in Fig. 1, a scan of a pH2 urine extract, and Fig. 2, a graphical representation of relative ion abundance in 10 amu segments from m/e 120-260.

The literature data on drug mass spectra, tabulated in Table I indicate that most drugs have molecular weights of 200-450 and produce abundant ions at m/e $>$150.

Thus, drugs can be analyzed in biological fluids by monitoring the interference-free high mass region.

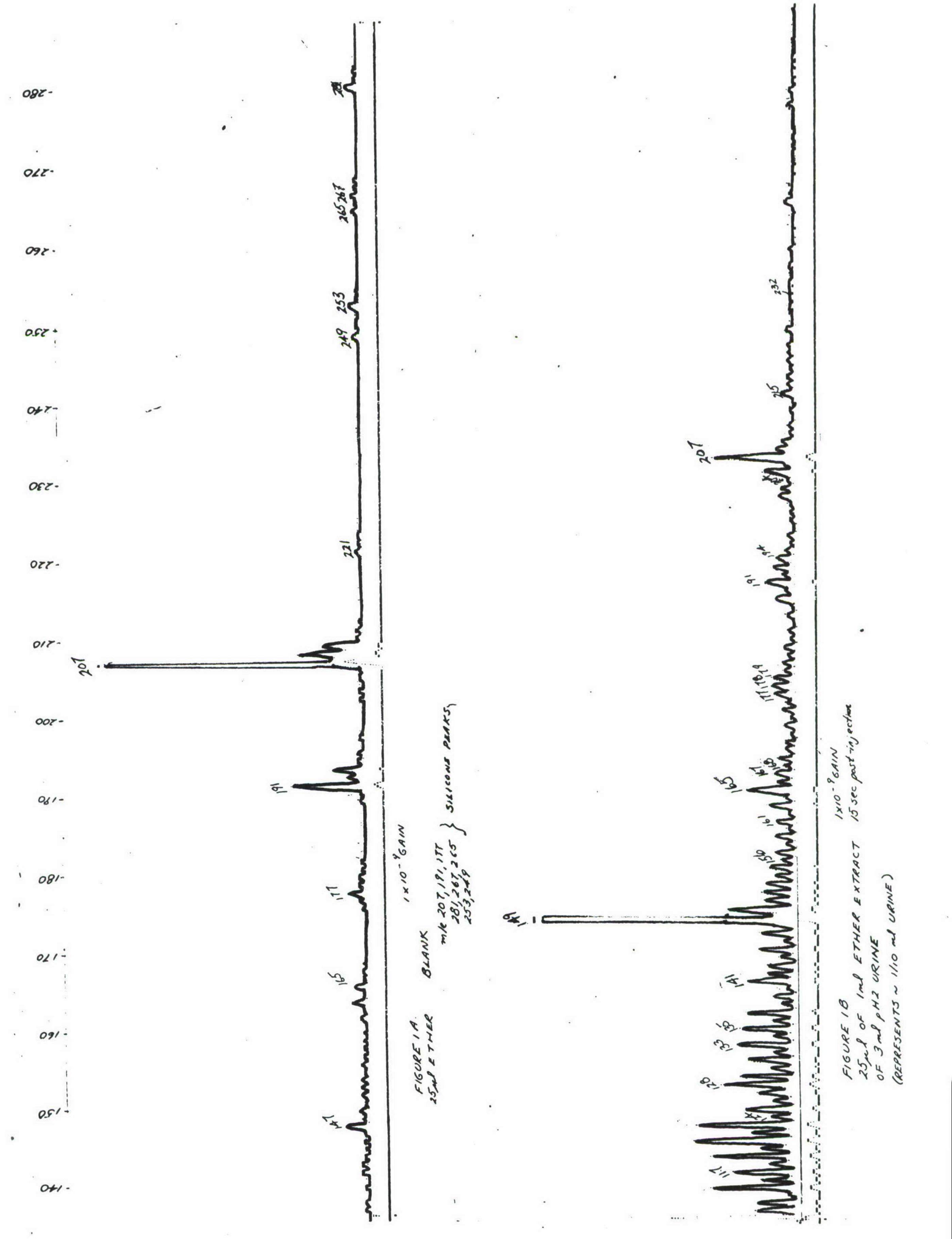


FIGURE 1A
25µl ETHER BLANK
m/e 207, 191, 177, 281, 267, 265, 253, 249 } SILICONE PEAKS
1x10^-9 GAIN

FIGURE 1B
25µl OF 1ml ETHER EXTRACT OF 3ml pH2 URINE
(REPRESENTS ~ 1/10 ml URINE)
1x10^-9 GAIN
15 sec post-injection

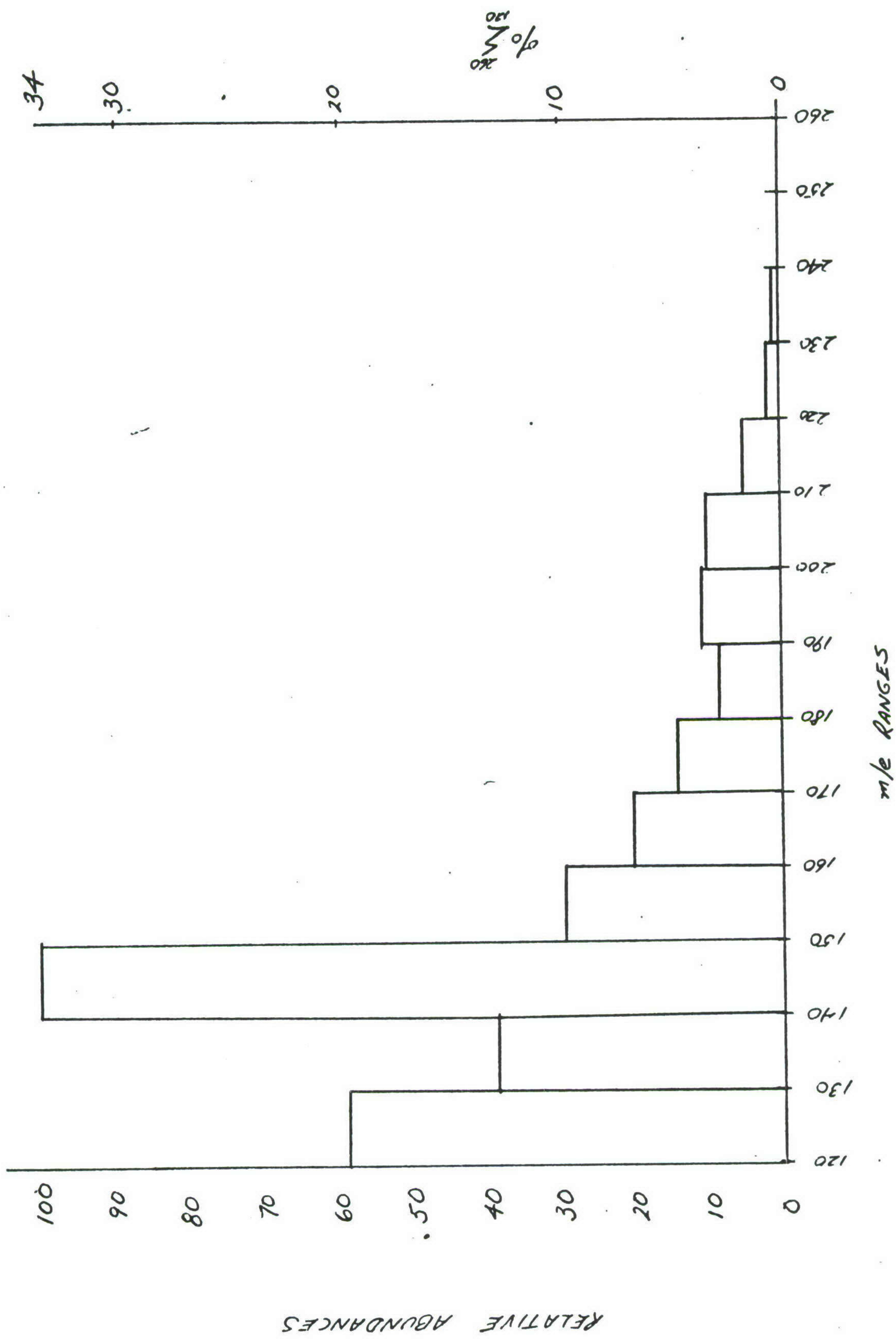
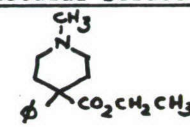
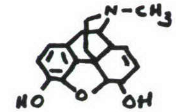
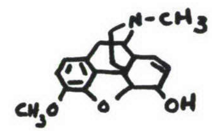
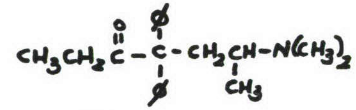
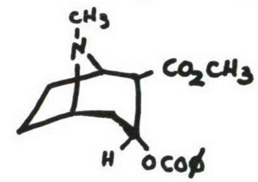
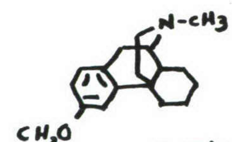
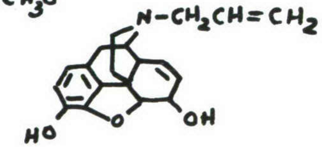


FIGURE 2.
ACID (pH2) EXTRACT OF .100 ml URINE (NO DRUGS TAKEN)

TABLE I. MASS SPECTRAL DATA OF COMMON ABUSE DRUGS

(A) Alkaloids, Narcotics   Basic Drugs

Drug	Eight Most intense Ions -Ion Relative Abundances								CVA-Utilizable Ions	$\%\sum_1^8$ *	Molecular Structure
Meperidine MW 247 MP of HCl Salt is 186°C	71 (100)	70 (56)	247 (38)	57 (35)	42 (34)	246 (32)	91 (24)	103 (21)	247-246	20%	
Morphine MW 285 MP of mono-hydrate 230°	285 (100)	162 (38)	215 (31)	42 (21)	286 (20)	124 (19)	284 (18)	174 (16)	286-285-284-215-174-162-124	92	
Codeine MW 299 MP 155°	299 (100)	162 (51)	229 (39)	42 (34)	214 (24)	300 (21)	124 (21)	188 (20)	300-299-229-	89	
Methadone MW 309 MP 78°	91 (100)	223 (81)	294 (81)	57 (80)	42 (77)	56 (77)	165 (76)	44 (65)	294-223-165	60	
Cocaine MW 303 MP 98°	82 (100)	28 (45)	182 (44)	83 (32)	77 (31)	42 (29)	94 (26)	105 (25)	182	13	
Dextromethorphan MW 271 MP of HBr Salt is 124-126°	59 (100)	42 (39)	150 (31)	271 (31)	44 (23)	171 (19)	115 (16)	128 (16)	271-171-150-128	35	
Nalorphine MW 311 MP 208-209°	271 (100)	44 (35)	270 (27)	214 (23)	272 (19)	42 (18)	43 (16)	70 (14)	272-271-270-214	67	

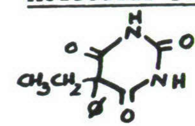
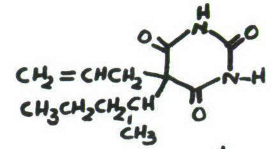
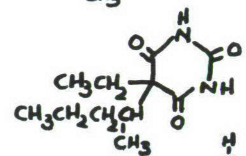
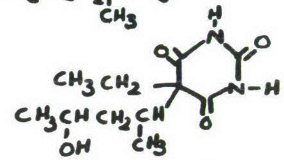
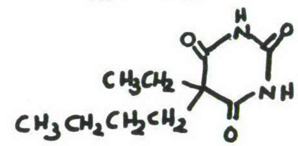
* $\%\sum_1^8$ = Abundance of CVA-Utilizable ions relative to total abundance of eight most intense ions.

**Molecular ion is underlined.

NOTE: Data from compilation by K. Biemann, MIT Department of Chemistry, Cambridge, Massachusetts

TABLE I. MASS SPECTRAL DATA OF COMMON ABUSE DRUGS

(B) Barbiturates Acidic Drugs

Drug	Eight Most Intense Ions Ion Relative Abundances								CVA-Utilizable Ions	$\%\sum_{1}^{8}$	Molecular Structure
Phenobarbital MW 232 MP 174-178°C	204 (100)	63 (34)	146 (34)	232 (28)	117 (22)	143 (18)	174 (18)	89 (17)	232-204-174-146	77%	
Secobarbital MW 238 MP 100°	41 (100)	168 (79)	167 (72)	43 (68)	39 (56)	55 (41)	97 (34)	124 (29)	168-167-124	61	
Pentobarbital MW 226 MP 130°	156 (100)	141 (55)	43 (30)	41 (22)	157 (22)	55 (10)	71 (7)	39 (7)	157-156-141	70	
Hydroxypen- tobarbital*	156 (100)	141 (58)	157 (43)	69 (37)	45 (33)	41 (23)	197 (21)	43 (15)	197-157-156-141	67	
Amobarbital MW 226 MP 156-158°	156	141	157	41	43	142	197	55	197-157-156-142	84	

*Metabolite of Pentobarbital

TABLE I. MASS SPECTRAL DATA OF COMMON ABUSE DRUGS

(C) Tranquilizers Basic Drugs

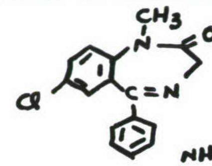
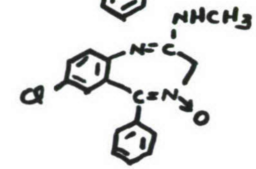
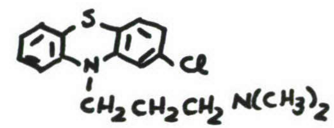
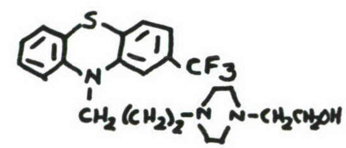
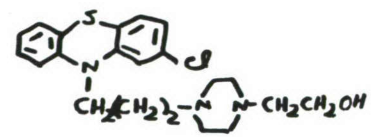
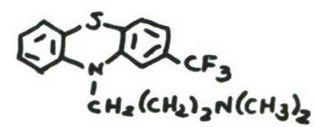
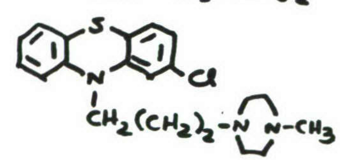
Drug	Eight Most Intense Ions Ion Relative Abundances								CVA-Utilizable Ions	$\% \sum_{1}^{8}$	Molecular Structure
Diazepam MW 284 MP 125-6°C	256 (100)	283 (75)	255 (60)	284 (59)	257 (48)	258 (38)	285 (34)	165 (31)	All	100%	
Chlordiazepoxide MW 299 MP 236-7°	282 (100)	283 (71)	284 (43)	299 (34)	285 (23)	77 (22)	56 (19)	41 (18)	299-285-284-283-282	82	
Chlorpromazine MW 318 Liq. BP 200-205 0.8	58 (100)	86 (26)	318 (23)	85 (12)	272 (9)	320 (8)	42 (6)	232 (5)	320-318-272-232	24	
Fluphenazine MW 437 Liq. BP 268-74 0.5	42 (100)	280 (100)	70 (96)	143 (96)	56 (95)	113 (85)	72 (80)	100 (79)	280-143	27	
Perphenazine MW 403 MP 94-100°	42 (100)	246 (66)	232 (59)	70 (49)	56 (36)	143 (36)	214 (28)	43 (20)	246-232-214-143	43	
Triflupromazine MW 352	58 (100)	86 (31)	352 (24)	85 (18)	306 (10)	59 (6)	266 (5)	248 (5)	352-306-266-248		
Prochlorperazine MW 373	113 (100)	70 (89)	373 (58)	141 (48)	43 (44)	42 (44)	44 (20)	375 (20)	375-373-141	30	

TABLE I. MASS SPECTRAL DATA OF COMMON ABUSE DRUGS

(C) Tranquilizers Basic Drugs

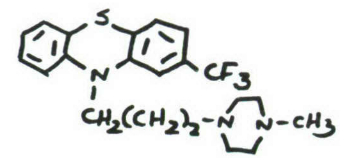
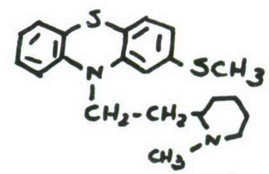
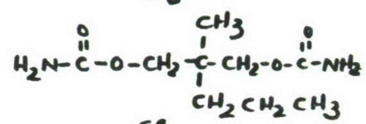
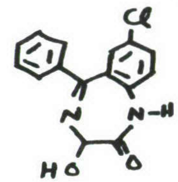
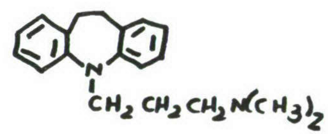
Drug	Eight Most Intense Ions Ion Relative Abundances								CVA-Utilizable Ions	% $\sum_{1}^{8}$	Molecular Structure
Trifluoperazine MW 407 MP of di-HCl Salt is 236-7^{o}	113 (100)	70 (81)	43 (56)	127 (45)	141 (44)	248 (44)	266 (42)	42 (38)	266-248-141 -127	40%	
Thioridazine MW 370	98 (100)	70 (13)	370 (9)	126 (8)	99 (7)	185 (4)	244 (3)	125 (3)	370-244-185 126-125	18	
Meprobamate MW 218 MP 104-6^{o}	83 (100)	55 (59)	71 (44)	96 (43)	114 (42)	144 (34)	62 (30)	56 (25)	144	9	
Oxazepam MW 286	257 (100)	259 (36)	77 (31)	229 (29)	104 (21)	286 (20)	241 (19)	214 (11)	286-259-257 241-229-214	80	
Imipramine MW 280	235 (100)	58 (78)	234 (64)	85 (62)	280 (48)	195 (29)	193 (23)	35 (20)	280-235-234 195-193	62	

TABLE I. MASS SPECTRAL DATA OF COMMON ABUSE DRUGS

(D) Hallucinogens — Basic Drugs

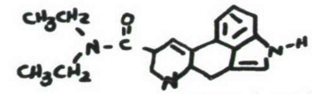
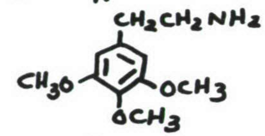
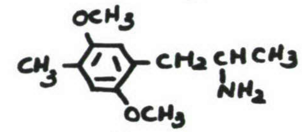
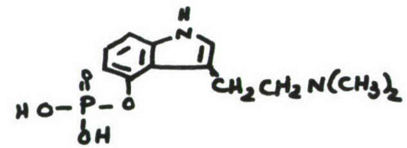
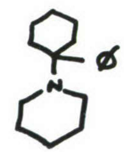
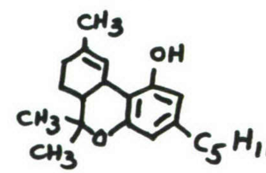
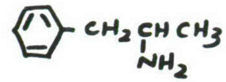
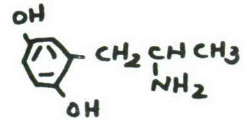
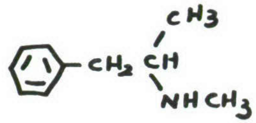
Drug	Eight Most Intense Ions -Ion Relative Abundances								CVA-Utilizable Ions	$\% \sum_{1}^{8}$	Molecular Structure
Lysergic Acid Diethylamide - 25 MW 323 MP 80-85°C	323 (100)	221 (64)	181 (41)	222 (36)	223 (32)	207 (29)	324 (22)	72 (21)	324-323-223-222-221-207-181	94%	
Mescaline MW 211 MP 35-6°	182 (100)	30 (88)	167 (54)	181 (49)	211 (21)	151 (18)	183 (13)	148 (11)	211-183-182 181-167-151-148	75	
STP (DOM) MW 209	44 (100)	166 (40)	151 (12)	57 (7)	43 (6)	91 (5)	129 (5)	209 (4)	209-166-151 129	38	
DMT MW 188 MP 45-46°	58 (100)	44 (21)	188 (5)	130 (5)	42 (4)	143 (4)	59 (2)	77 (2)	188-143-130	7	
Psilocybin MW 284 MP 220-8°	58 (100)	204 (15)	59 (3)	146 (3)	159 (2)	205 (2)	160 (1)	57 (1)	205-204-160 159-146	17	
Phencyclidine MW 243 MP of HCl Salt is 228-9°	200 (100)	91 (66)	84 (47)	28 (46)	242 (36)	243 (31)	115 (30)	129 (29)	243-242-200 -129	54	
Tetrahydrocannabinol MW 314 Liq. BP 155-7 (.05)	314 (100)	299 (80)	231 (55)	271 (41)	43 (36)	41 (35)	315 (24)	243 (22)	315-314-299-271 243-231	82	

TABLE I. MASS SPECTRAL DATA OF COMMON ABUSE DRUGS

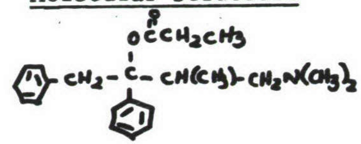
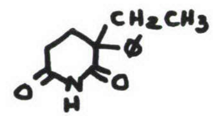
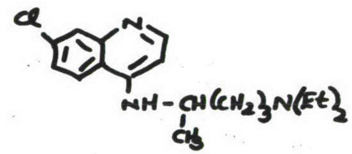
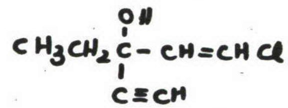
(E) Amphetamines Basic Drugs

Drug	Eight Most Intense Ions -Ion Relative Abundances								CVA-Utilizable Ions	% $_{1}^{8}$	Molecular Structure
Amphetamine MW 135 Liq. BP 200-203^{o}	44 (100)	91 (8)	65 (4)	42 (3)	45 (3)	120 (3)	40 (2)	92 (2)	120	21%	
2, 5-Dimethoxy Amphetamine MW 167	44 (100)	152 (21)	28 (12)	137 (8)	77 (6)	65 (5)	91 (5)	78 (4)	152-137	18	
Methylamphetamine MW 149	58 (100)	91 (7)	59 (5)	43 (3)	56 (3)	65 (3)	134 (3)	39 (2)	134	2	

See STP under Hallucinogens

TABLE I. MASS SPECTRAL DATA OF COMMON ABUSE DRUGS

(F) Miscellaneous

Drug	Eight Most Intense Ions -Ion Relative Abundances								CVA-Utilizable Ions	% $\sum_{1}^{8}$	Molecular Structure
Propoxyphene MW 339 MP of HCl Salt is 170-1^{o}C	58 (100)	57 (4)	59 (4)	29 (4)	49 (4)	91 (4)	42 (3)	105 (1)	None	0%	
Propoxyphene Metabolite	44 (100)	220 (92)	100 (62)	57 (42)	205 (35)	91 (26)	129 (20)	221 (20)	221-220-205 -129	42	
Glutethimide MW 217	117 (100)	189 (88)	132 (55)	115 (51)	91 (47)	160 (44)	77 (30)	39 (28)	189-160-132	42	
Chloroquine MW 319	86 (100)	58 (10)	73 (5)	87 (5)	319 (5)	41 (4)	99 (4)	245 (4)	319-245	7	
Ethchlorvynol MW 145	115 (100)	117 (29)	89 (24)	53 (20)	109 (15)	51 (13)	91 (12)	39 (8)	None	0	

III. CVA Mass Spectrometry of Standard Solutions of Drugs

Representative drugs (underlined) were chosen to test the above concept:

(1) The barbiturates, pheno-, seco- and pentobarbital, are the most commonly encountered drugs in the poisoning circumstance. Phenobarbital is often used to "cut" heroin. Secobarbital ("reds") abuse is an increasing street problem.

(2) The tranquilizer diazepam is the number-one selling tranquilizer in the United States. It constitutes a serious alcohol-interaction poisoning problem. Its use is detected in urine by analysis for the metabolite oxazepam. Oxazepam itself is a tranquilizer.

(3) The narcotic analgesic heroin is the major drug problem in the Army. Heroin abuse is detected by analysis of its metabolite morphine. Methadone abuse is increasing as its availability through addict maintenance programs increases. Codeine is a commonly used narcotic analgesic which is partially metabolized to morphine and its use could constitute a mistaken conclusion of heroin abuse (see Section VII). Codeine was shown to be a "marker" of heroin addiction (Section VII) in this study.

(4) Cocaine is an increasing street drug problem. It is occasionally mixed with heroin.

(5) Glutethimide, a commonly used sedative, constitutes a serious poisoning problem. Its use is often detected by analysis for the urinary metabolite, α-phenylglutarimide.

(6) Mescaline, a hallucinogen, is currently a low-level abuse problem, but may be a potentially serious problem. It is often detected by analysis for the urinary metabolite trimethoxyphenylacetic acid.

(7) Chloroquine is an anti-malarial drug which constitutes a compliance problem to the Army, due to the drug's gastro-intestinal side effects

(8) Sulfamethazine is a commonly encountered anti-microbial agent.

CVA-spectra demonstrating approximately 100 ng-1 μg sensitivity for standard solutions of these drugs are shown in Figures 3-13 and are discussed below.

The abundant ions expected for the barbiturates based on literature data,[3, 4] are observed in the CVA spectrum of Fig. 3:

[3] R. T. Coutts and R. A. Locock, J. Pharm Sci. 57, 2096 (1968).

[4] Table I data.

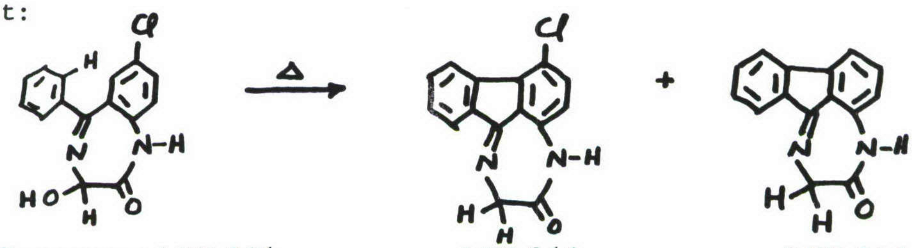
m/e 204, 161, 146	phenobarbital
m/e 167, 168, 124	secobarbital
m/e 156, 141	pentobarbital

Relative peak abundances are also similar to those in the literature. The barbiturates were run simultaneously as they are frequently abused in concert. Comparison of Fig. 3 to Fig. 1 indicates that CVA analysis of sub-microgram barbiturate levels in urinary acidic extracts is feasible. Similar feasibility is indicated for the other drugs studied.

As seen in Fig. 4, 2 µg diazepam produced a signal to background peak of X350 at m/e 283. This indicates a detection limit of ≲100 ng. The mass peaks observed (m/e 151, 152, 165, 177, 193, 219, 221, 228, 241, 256, 283) are identical to the literature mass spectrum[5], indicating negligible decomposition in the CVA-inlet-source system. Diazepam has been shown to suffer thermolytic degradation on GC columns, necessitating derivative formation in GC work.

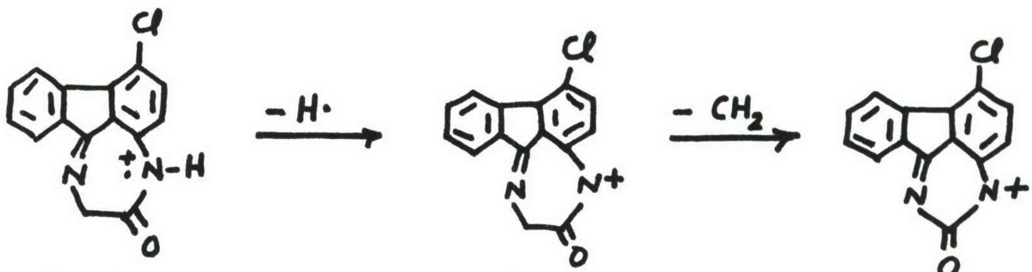
The one case of thermal decomposition observed was that of oxazepam, a tranquilizer and also the major urinary metabolite of diazepam. The CVA-mass spectrum (Fig. 5) does not contain either the expected molecular ion of oxazepam at m/e 286, the base peak at m/e 257 (M-CO) or other major fragments.[5] The mass peaks observed (m/e 267, 253-base, 239, 233, 219, 205) indicate thermal decomposition in the inlet:

(1)



Oxazepam MW 286 MW 268 MW 234

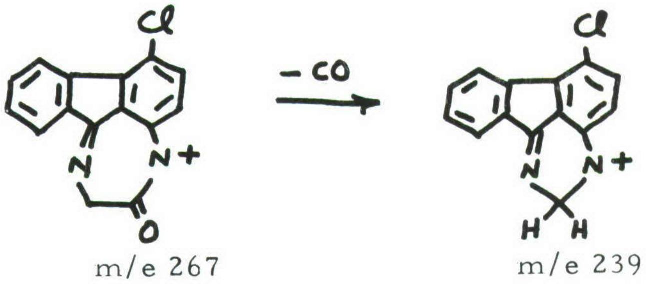
(2)



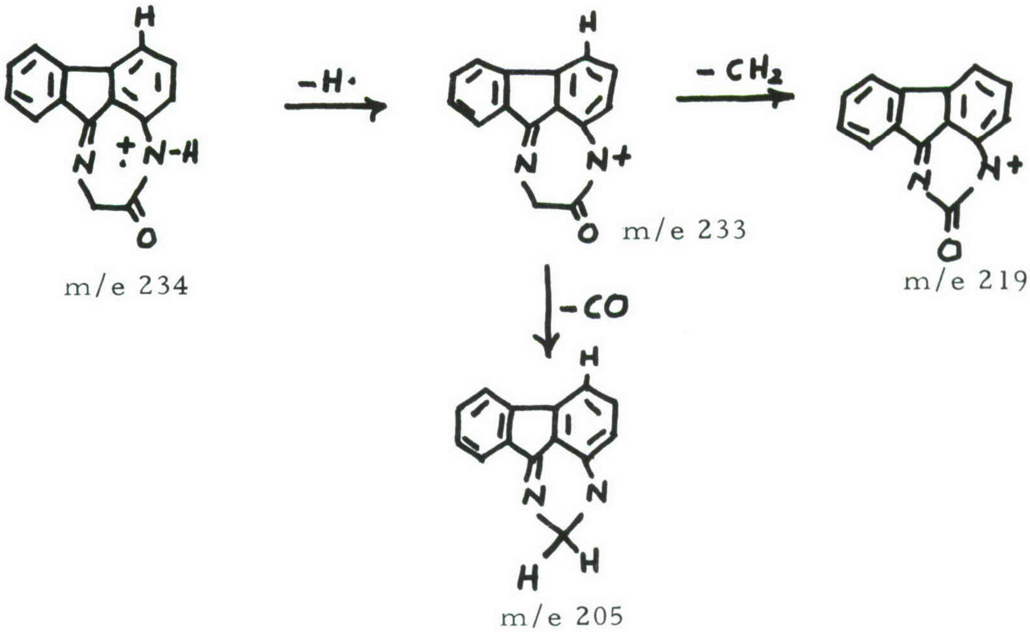
m/e 268 m/e 267 m/e 253

[5]W. Sadée, J. Med. Chem. 13, 475 (1970).

(3)



(4)



Literature references on the mass spectra of the drugs studied are presented in Appendix I. The commercial vs. generic names of the drugs are presented in Appendix II. Metabolic pathways of the drugs studied are presented in Appendix III.

In screening for oxazepam one would therefore utilize the observed mass peaks of its decomposition products. As seen in Fig. 5B, the detection limit based on the mass peak at m/e 253 is $\leq$ 100 ng.

Abbreviated CVA mass spectra of the remaining drugs and/or metabolites are presented in Figs. 6-13. Sub-microgram sensitivity is indicated for the major high mass peak in all cases.

IV. Therapeutic Drug Level Analysis

Drug levels found in a user's body fluids depend on disposition, metabolism, and excretion characteristics of the particular drug, and on the drug dose. Three categories of drug dosage can be defined: therapeutic, addictive-abuse, overdose. These categories are discussed below for several representative drugs.

A therapeutic (analgesic) dose of heroin is 2-8 mg. In the U.S., however, heroin is not administered therapeutically. Abuse of heroin leads to tolerance development. The range of heroin dosage taken by heroin addicts is approximately 100 mg to 4000 mg per day. The overdose range is dependent on the level of tolerance of the user and can be any dosage greater than 50 mg. It is notable that for a drug characterized by tolerance development, abuse and overdose levels overlap.

Heroin is metabolized to morphine. Approximately 40-69% of dose is excreted in the urine in 24 hours, as morphine and morphine glucuronide. Thus, for a 100 mg dose and a 2 liter urine volume per 24 hours, total morphine level will be 50 mg/2 liters or 2.5 mg % (mg drug per 100 ml fluid). CVA sensitivity to morphine is approximately 500 ng. A 2.5 mg % urinary morphine level is equivalent to 500 ng/20 ul. Sample volumes up to 250 ul urine have been run successfully on the CVA system. Urinary morphine levels below 1 mg % can be readily detected by analyzing a concentrated urine extract (i.e., chloroform extract of hydrolyzed urine). This was demonstrated in the work at San Francisco General Hospital, as described in Section VII.

Similar calculations predict feasibility of CVA detection of therapeutic, abuse, and overdose drug levels in urine for the abuse drugs. Examples of drug dosage are:

(mg % = mg drug per 100 ml fluid)

Drug	Dose (mg/day) Therapeutic	Dose (mg/day) Abuse	Overdose
Secobarbital	100-200	200-2000	>200
Morphine	10	100-4000	>100
Methadone	60-100 (heroin withdrawal dose)	60- ?	> 60
Chlorpromazine	300-2000	300- ?	>1000
Diazepam	2-40	?	> 100

Instrument sensitivity of less than one microgram has been demonstrated for the above drugs and those discussed on page 16. Sensitivity was determined based on detection of 1 μg drug at a specific high mass ion (i.e., m/e 285 for morphine), with S/N >2.0. In all cases S/N was >5.0. Absolute detection limits were not pursued during this portion of the study for the following reasons:

(1) 1 μg detection was felt sufficient for initial urine and blood screening.

(2) Absolute detection limit should be determined using computer-driven specific ion-monitoring and data time-averaging to enhance S/N. Programming the computer for time-averaging was not within the scope of this contract.

Detection of methadone in a maintenance clinic urine (therapeutic dose) is described in Section IX.

Detection of chlorpromazine and of diazepam in urines of patients on therapeutic dosage are shown in Figures 14-15.

Analysis of urine from normal subjects (Varian personnel) following aspirin ingestion was undertaken to demonstrate that (1) aspirin (acetylsalicylic acid) and its major metabolite (salicylic acid) can be detected in urine after therapeutic dosage, and (2) the relatively low mass ions due to this commonly used drug and its major metabolite will not interfere with detection of the abuse drugs of interest (relatively high mass characteristic ions).

Aspirin (ASA) is hydrolyzed in the body to salicylic acid (SA). SA is then either excreted unchanged (~ 25% of dose) or as its metabolites: gentisic acid (4-8%), salicyluric acid (~50%), salicyl acyl and phenolic glucuronides (20-25%). ASA metabolism is shown in Figure 15B.

The level and distribution of these species are dependent on dose, time between dosage and urine collection, urinary pH, individual metabolism.

CVA analysis readily detects SA in urine, as evidenced by the characteristic m/e 138 and 120 peaks in Figure 15C and 15D. In cases where SA level was low, acid hydrolysis of the urine generated free SA (either from glucuronide or urate) which was then detected by CVA.

Another ASA metabolite, giving strong peaks at m/e 137, 120 and 92 was detected from some patient urines (Figure 15E). Salicyluric acid is suspected.

A CVA spectrum of SA in 10^{-2} M HCl did not detect the m/e 138, 120 peaks of SA. However, SA in 3 M HCl was readily detected. This phenomenon is unexpected since in 10^{-2} M HCl SA should be in the neutral form.

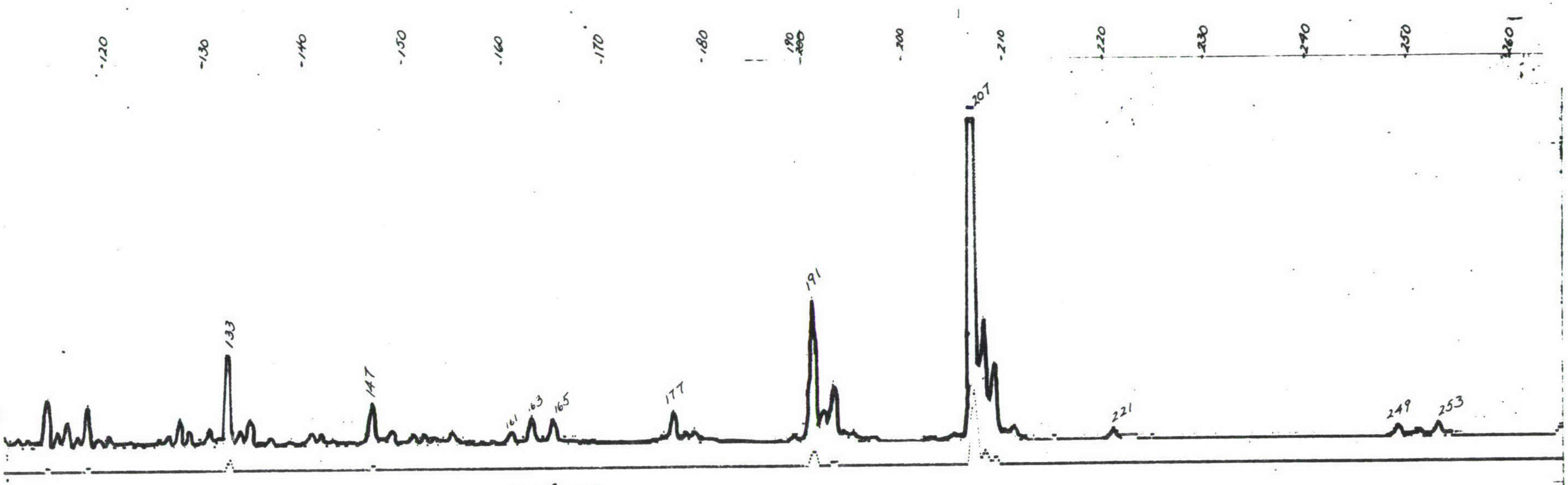


FIGURE 3A
1×10^{-9} GAIN
10 µl pH 2 AQ HCl BLANK
T_i 275 T_m 180

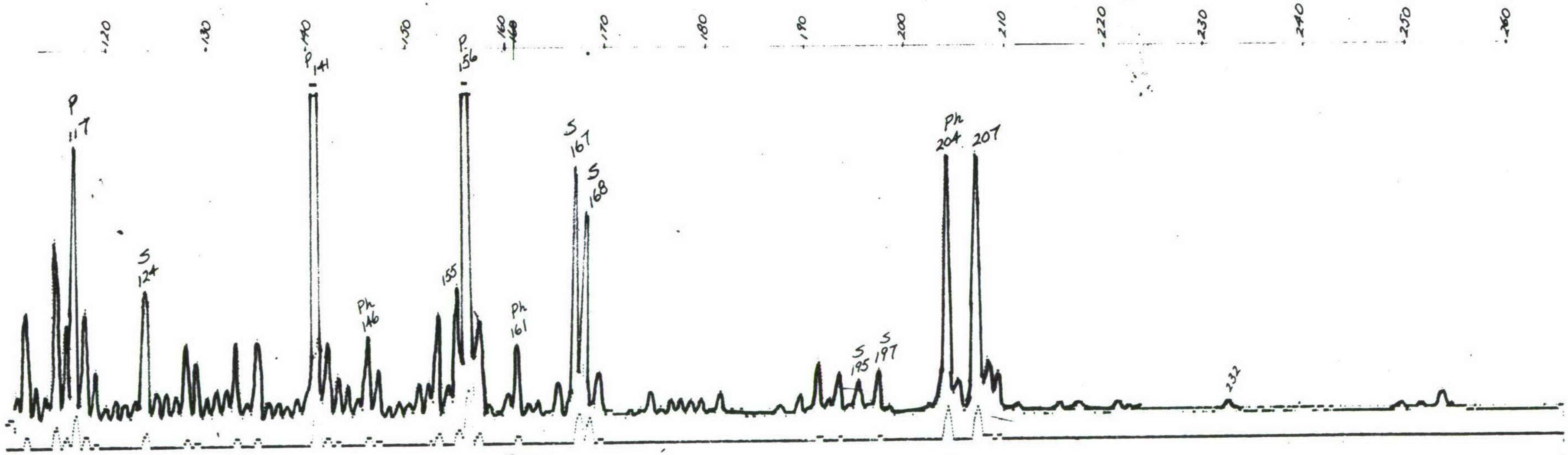


FIGURE 3B.
1×10^{-9} GAIN
15 sec post-injection
1 µg / 10 µl pH 2 AQ HCl
PHENOBARBITAL (Ph)
PENTOBARBITAL (P)
SECOBARBITAL (S)

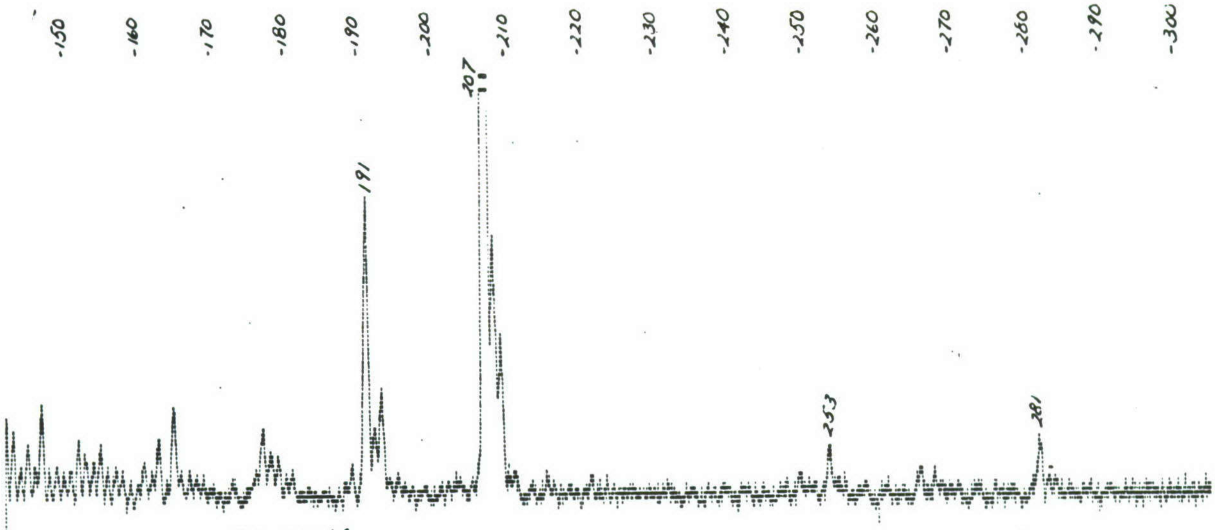


FIGURE 4A
10μl ACETONE BLANK

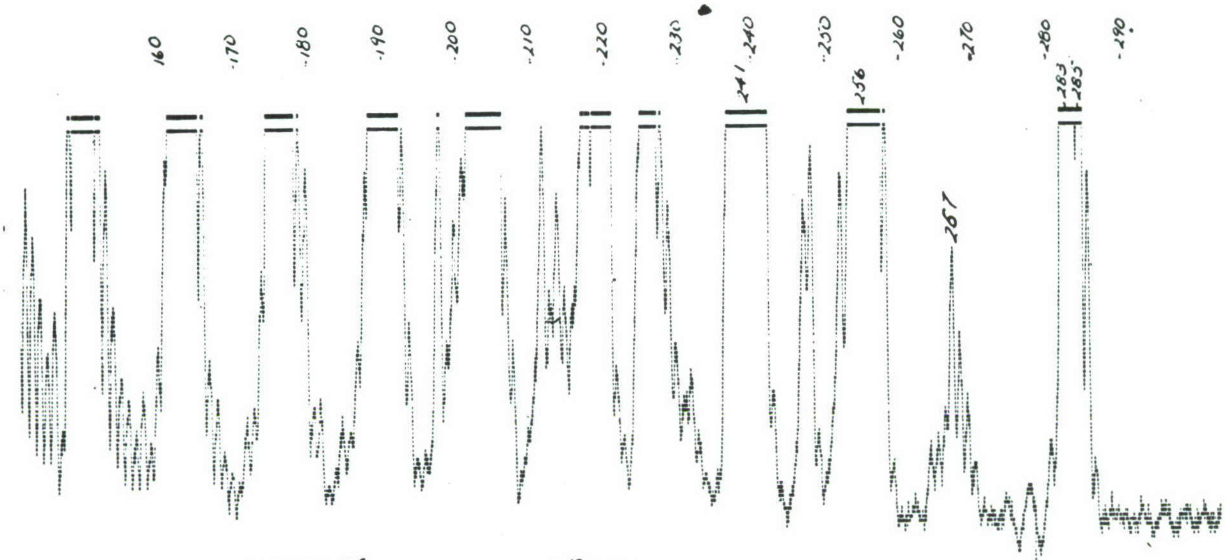


FIGURE 4B
2μg DIAZEPAM
IN 10μl ACETONE

1x10^-10 GAIN
15 SEC POST-INJECTION
PEAK MAXIMA

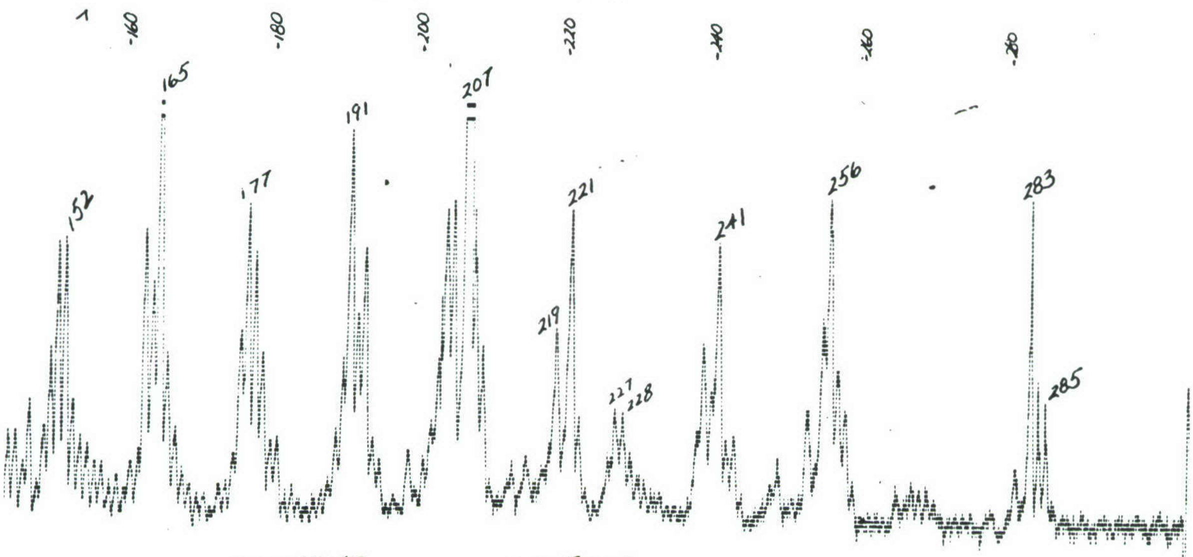


FIGURE 4C
2μg DIAZEPAM
IN 10μl ACETONE

1x10^-10 GAIN
$2\frac{1}{2}$ MIN POST-INJECTION

m/e 283 ~ $\frac{1}{15}$ x INTENSITY AT MAX SHOWN IN FIGURE } ∴ BKGD: 2/16"
PEAK MAX (m/e 283) = $15 \times \frac{47}{16} = \frac{705}{16}$"
5/8 (2μg) ≈ x 350

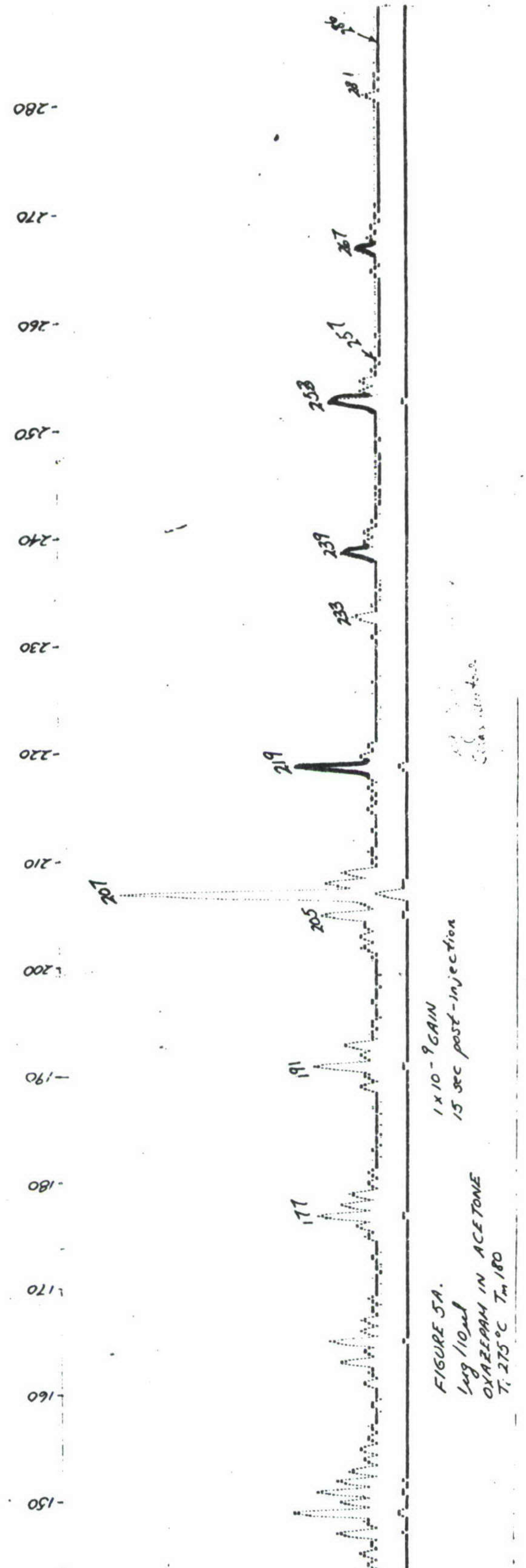


FIGURE 5A.
1µg/10µl
OXAZEPAM IN ACETONE
Ti 275°C Tm 180

1x10^-9 GAIN
15 sec post-injection

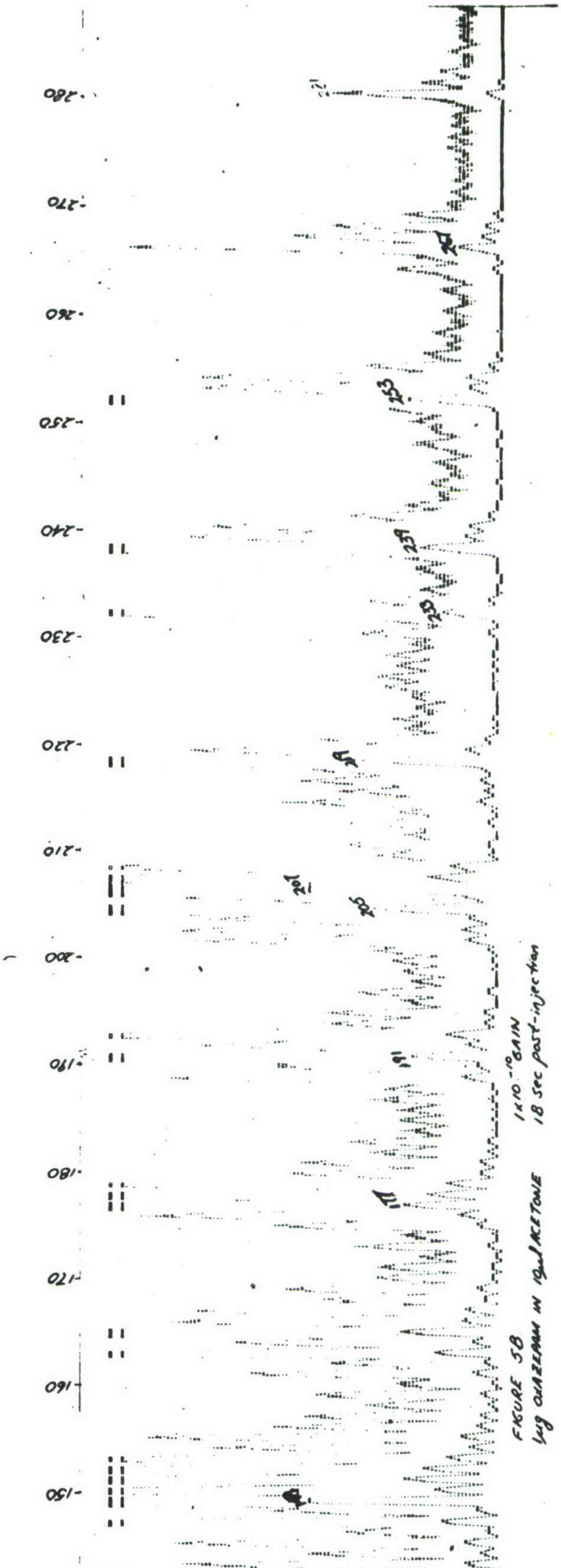


FIGURE 5B
1µg OXAZEPAM IN 10µl ACETONE

1x10^-10 GAIN
18 sec post-injection

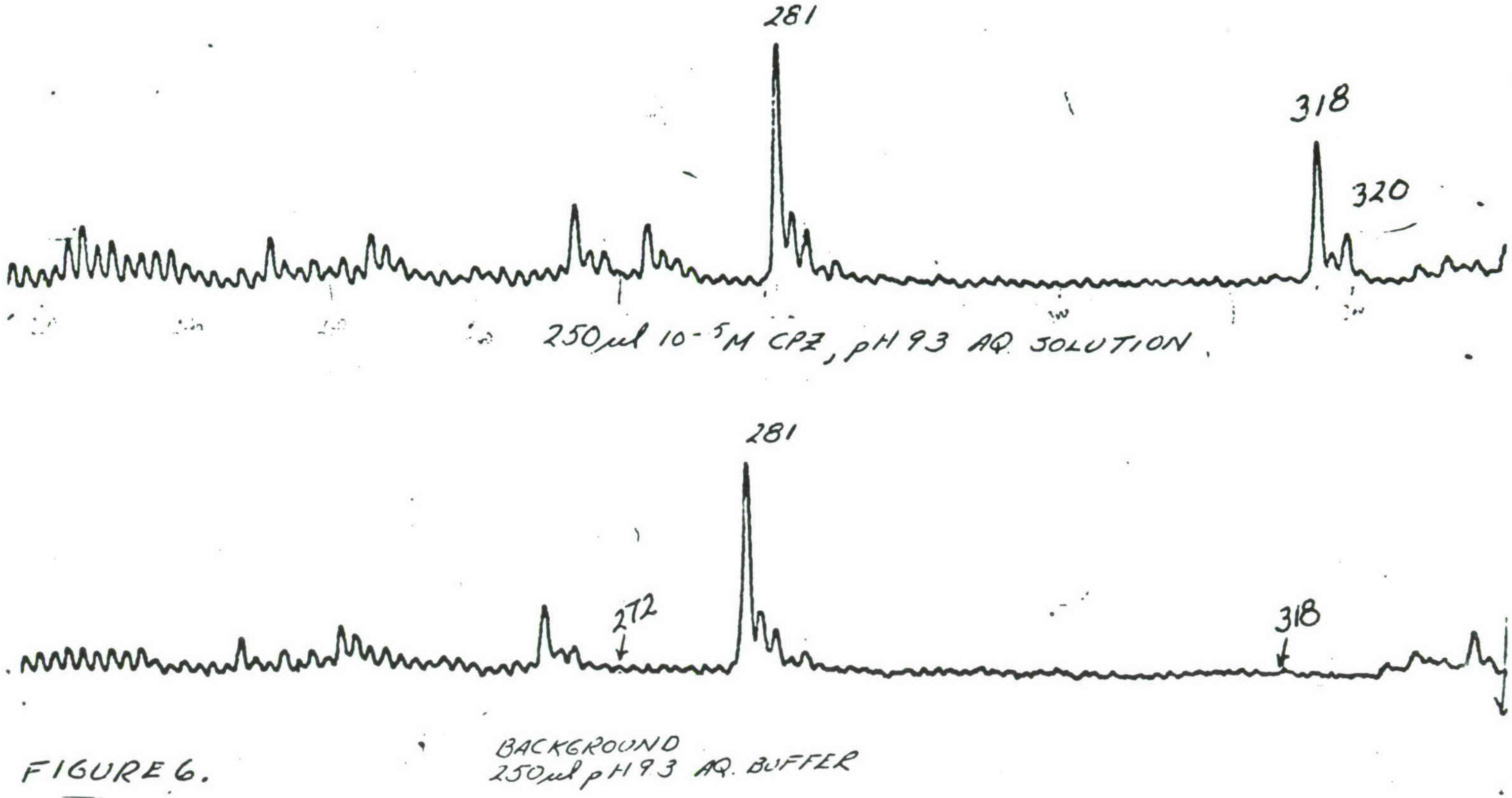


FIGURE 6.

CVA MASS SPECTRUM

10^{-5}M CHLORPROMAZINE IN AQUEOUS, BUFFERED SOLUTION

250μl INJECTION

∴ 0.8 μg

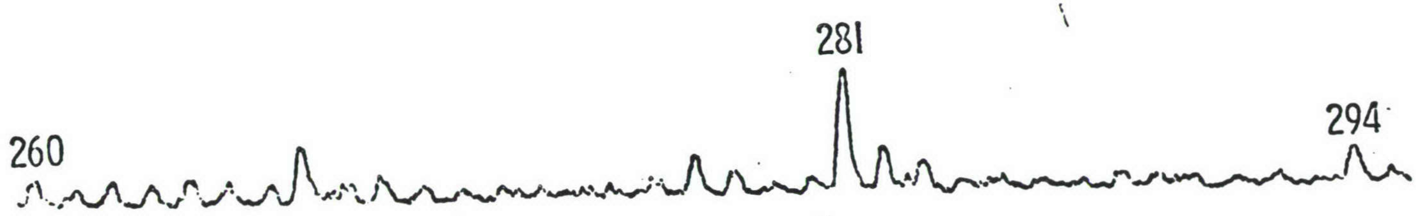


FIGURE 7. CVA SPECTRUM: 10^{-5}M - METHADONE IN PH 9.3 AQ. BUFFER
250 μl INJECTION, ∴ 0.8 μg
m/e 294 ⇒ METHADONE

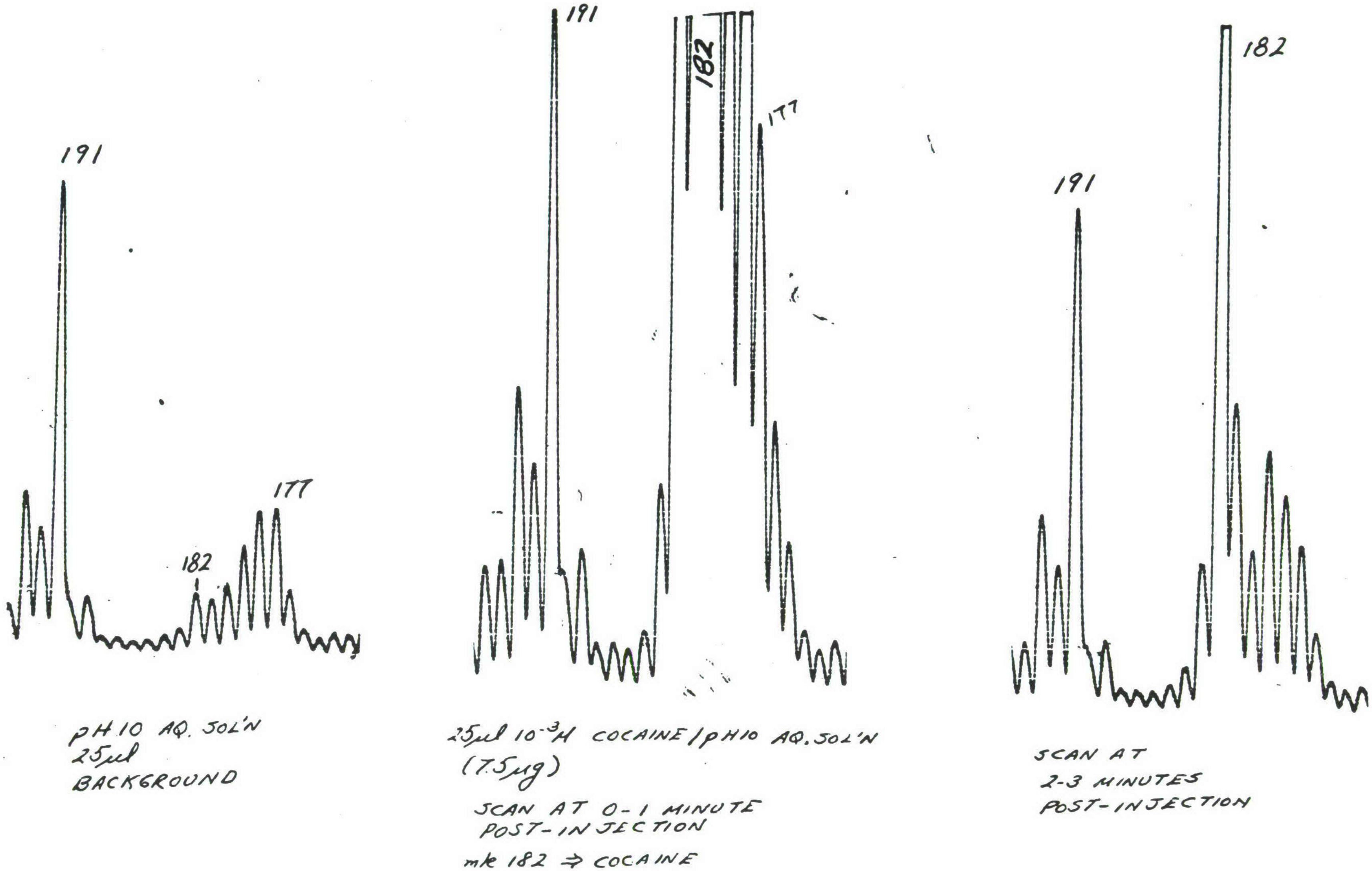
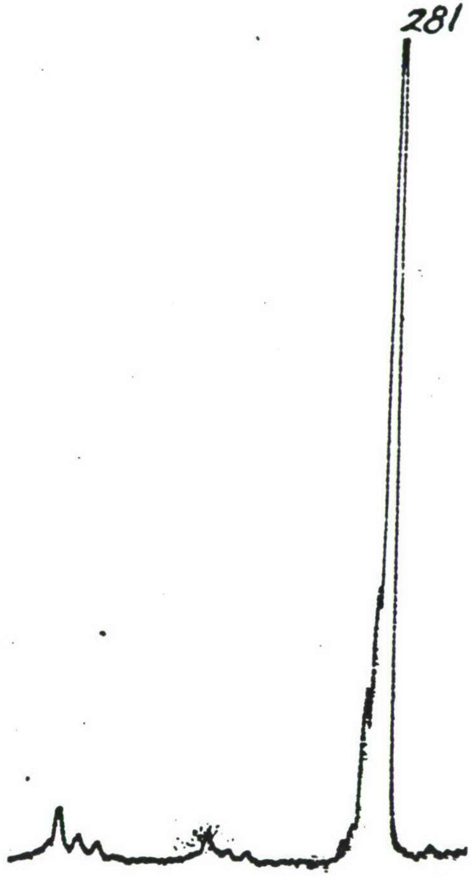
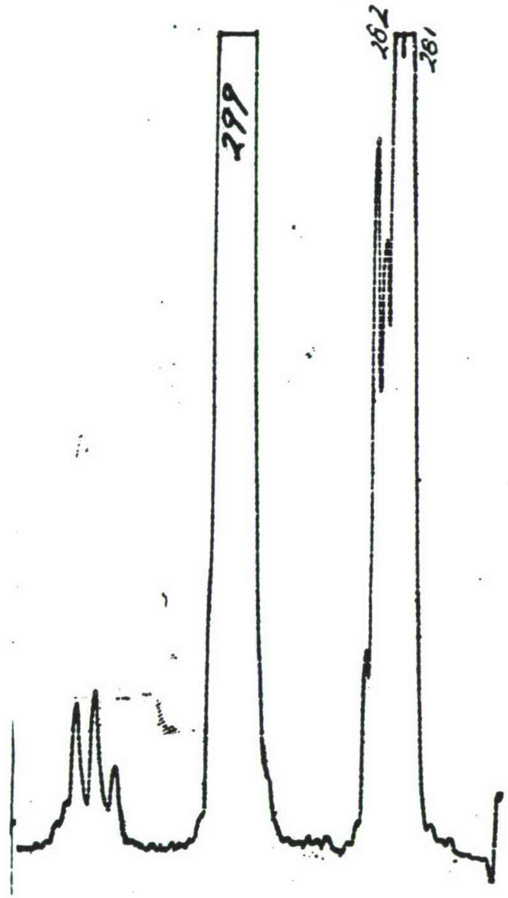


FIGURE 8.

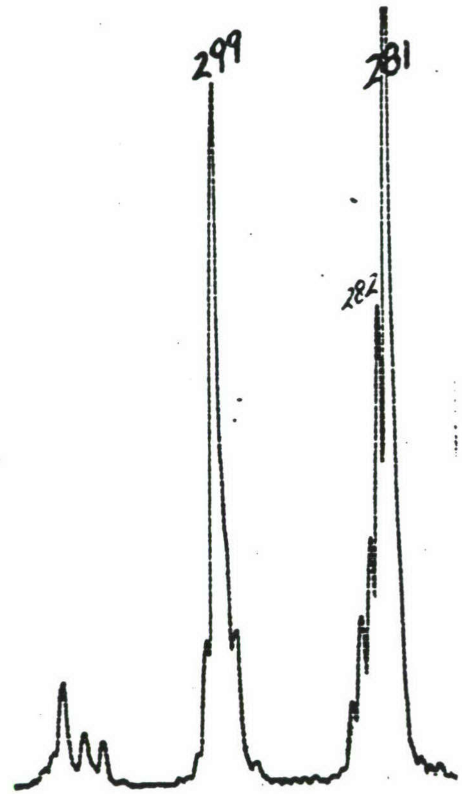
CVA MASS SPECTRUM OF AQUEOUS SOLUTION OF COCAINE



25μl AQ. pH 9.3 SOL'N
m/e 281 ⇒ SILICONE PEAK

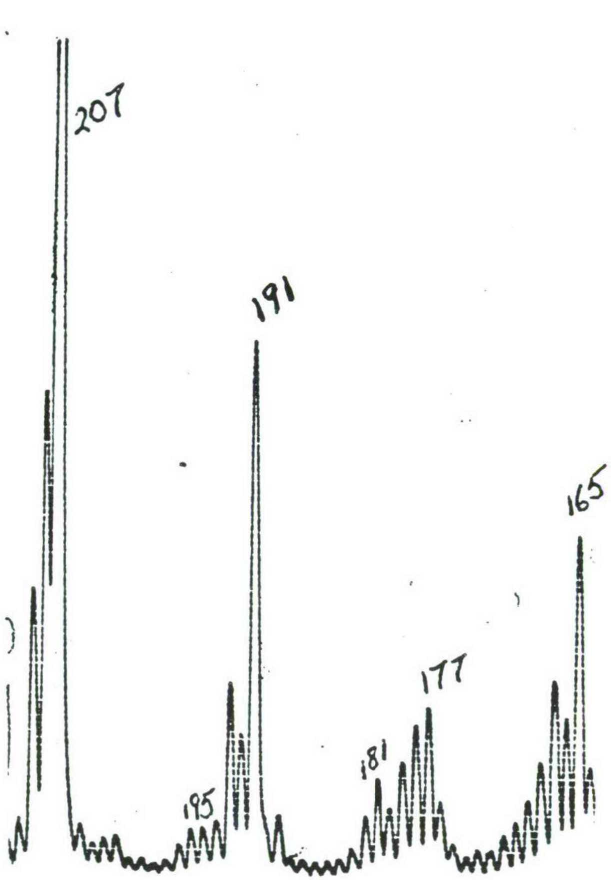


25μl 10^{-3}M CODEINE/AQ. pH 9.3 SOL'N
(∴ 6μg)
SCAN 0-1 MINUTE
m/e 299, 282 ⇒ CODEINE

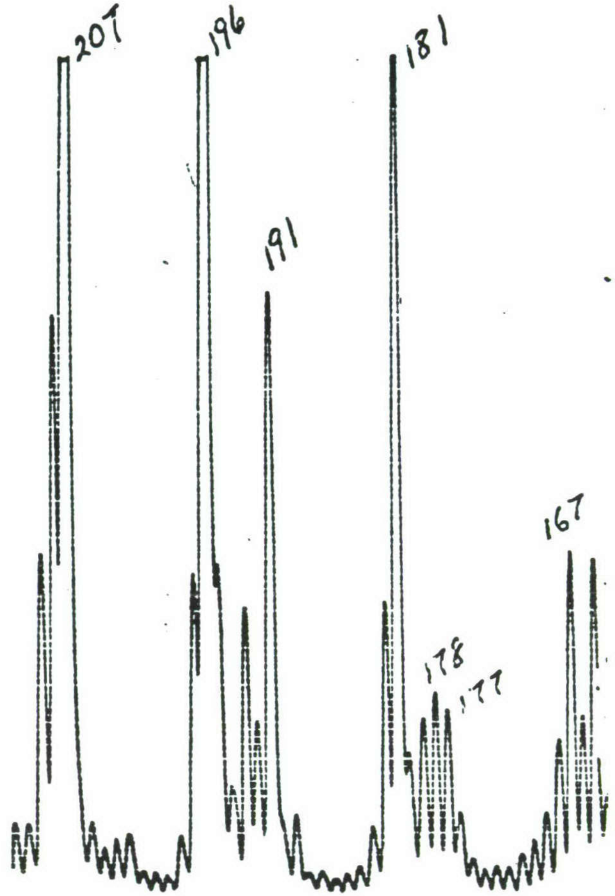


SCAN 4-5 MINUTES

CVA MASS SPECTRUM
6μg CODEINE
FIGURE 9.



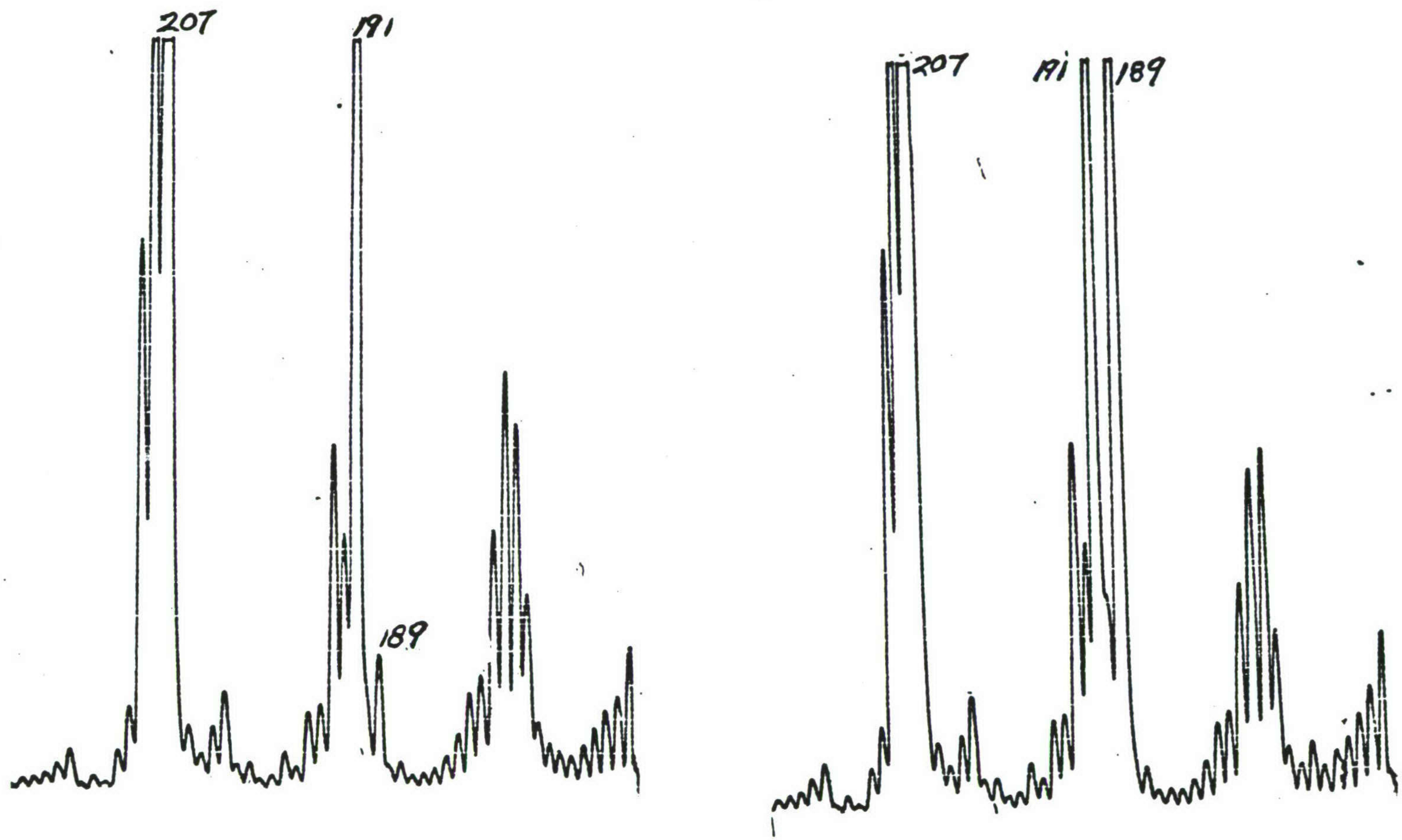
25µl AQ pH2 SOL'N
BACKGROUND



25µl 10^{-3}M TMPAA/pH2 AQ. SOL'N
m/e 196, 181 ⇒ TMPAA
(5µg)

FIGURE 10.

CVA MASS SPECTRUM
5µg TRIMETHOXYPHENYL ACETIC ACID
(URINARY METABOLITE OF MESCALINE)



25μl AQ. pH2 SOL'N
BACKGROUND

25μl 10^{-3}M ALPHA-PHENYLGLUTARIMIDE
IN AQ. pH2 SOL'N
(5μg)
m/e 189 ⇒ ALPHA-PHENYLGLUTARIMIDE

FIGURE 11.

CVA MASS SPECTRUM
5μg ALPHA-PHENYLGLUTARIMIDE
(URINARY METABOLITE OF DORIDEN)

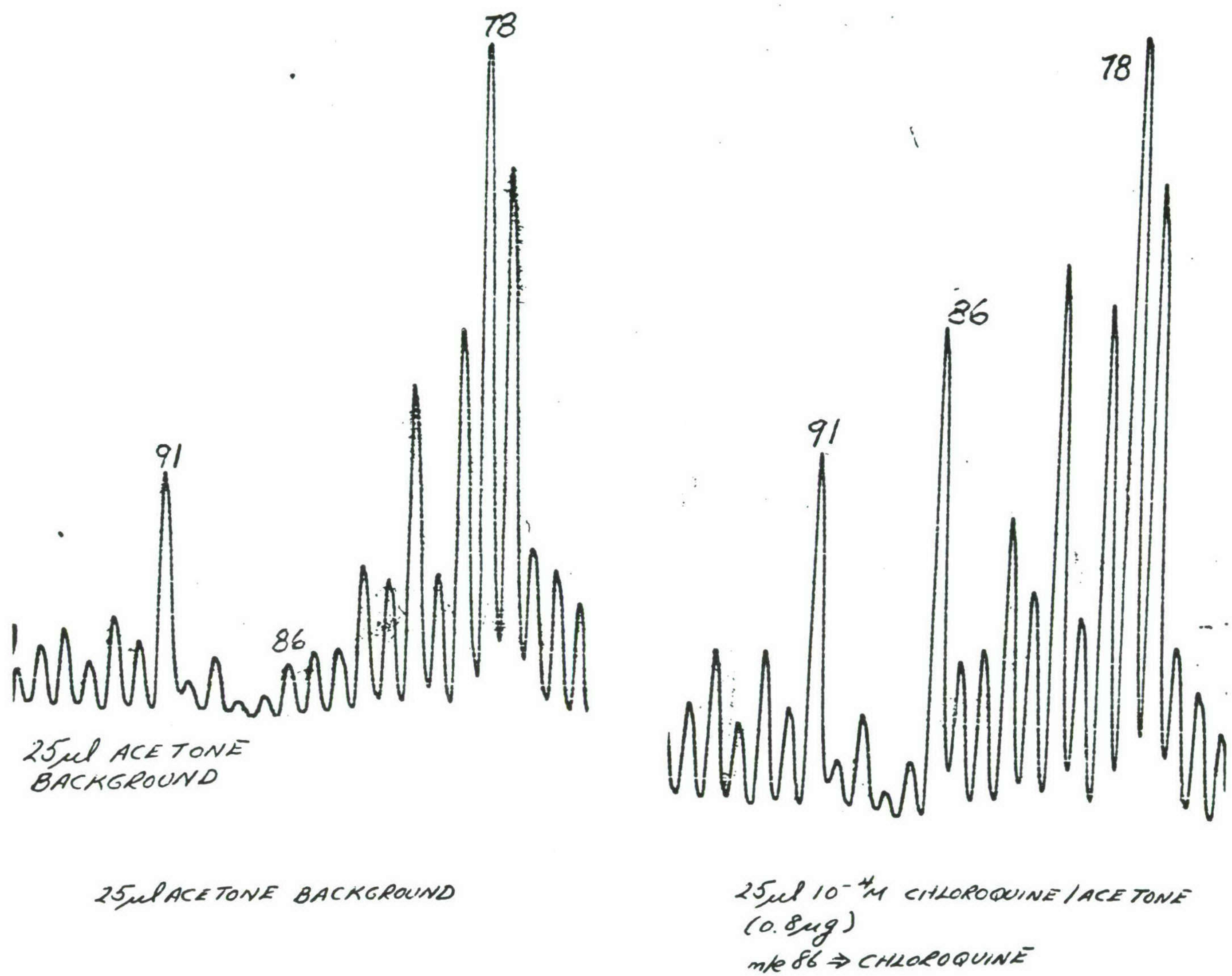
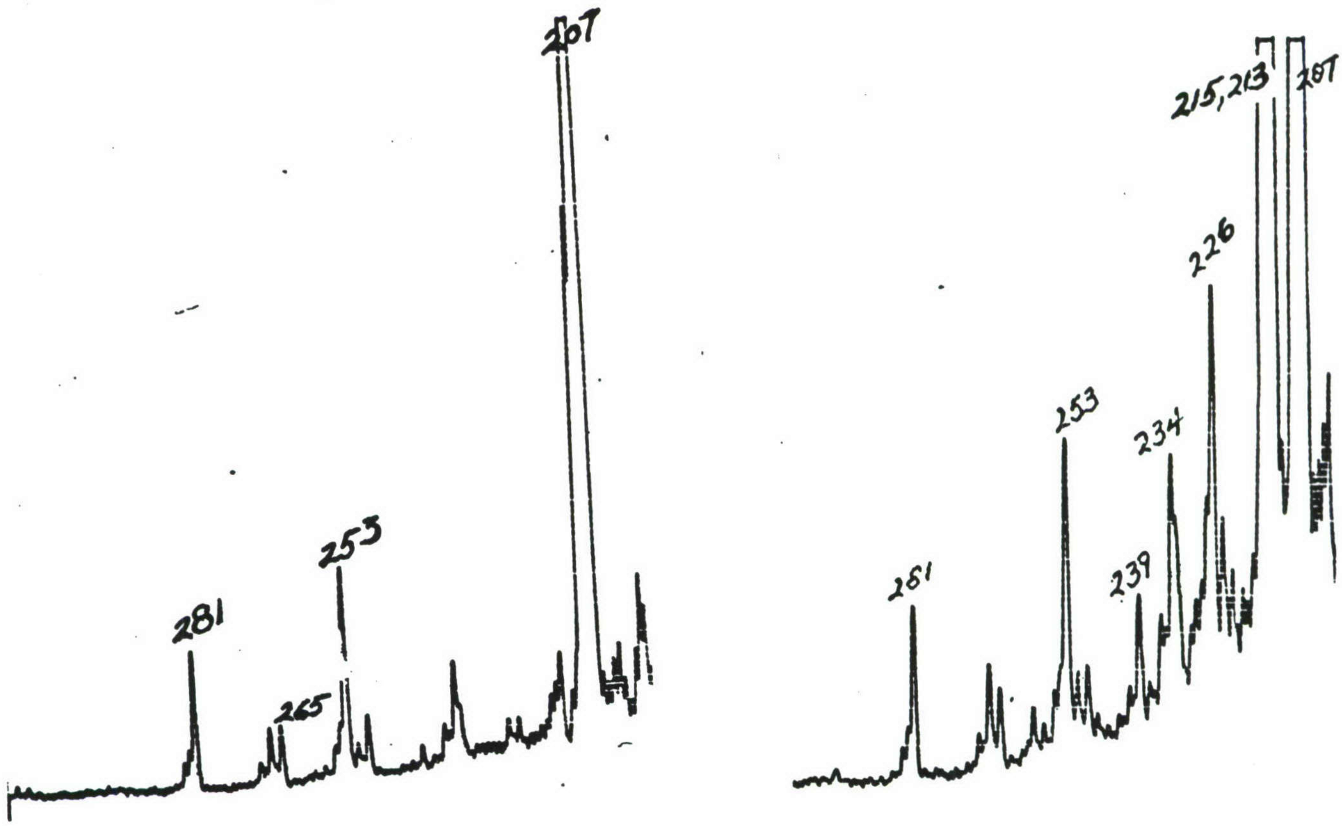


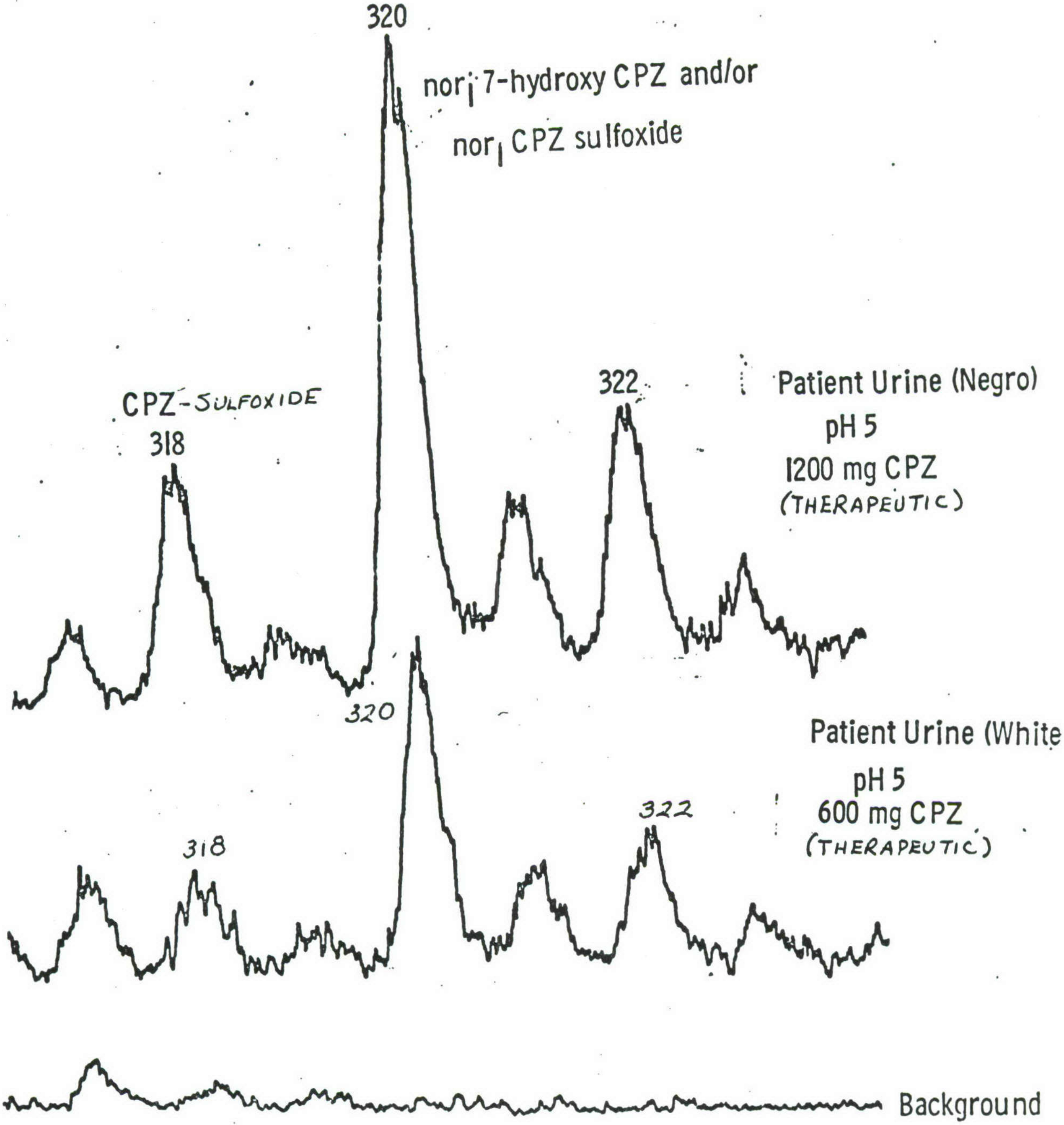
FIGURE 12.
CVA MASS SPECTRUM
0.8μg CHLOROQUINE



25µl DICHLOROMETHANE BACKGROUND

25µl 10^{-3}M SULFAMETHAZINE (7µg) IN DICHLOROMETHANE
m/e 213, 215 ⇒ SULFAMETHAZINE

FIGURE 13.
CVA MASS SPECTRUM
7µg SULFAMETHAZINE



ANALYSIS OF CHLORPROMAZINE METABOLITES IN PATIENT URINES

FIGURE 14. A. AQUEOUS BACKGROUND
B. 250μl PATIENT URINE - pH5 (NO ADJUSTMENT)
C. 250μl PATIENT URINE - pH5 (NO ADJUSTMENT)

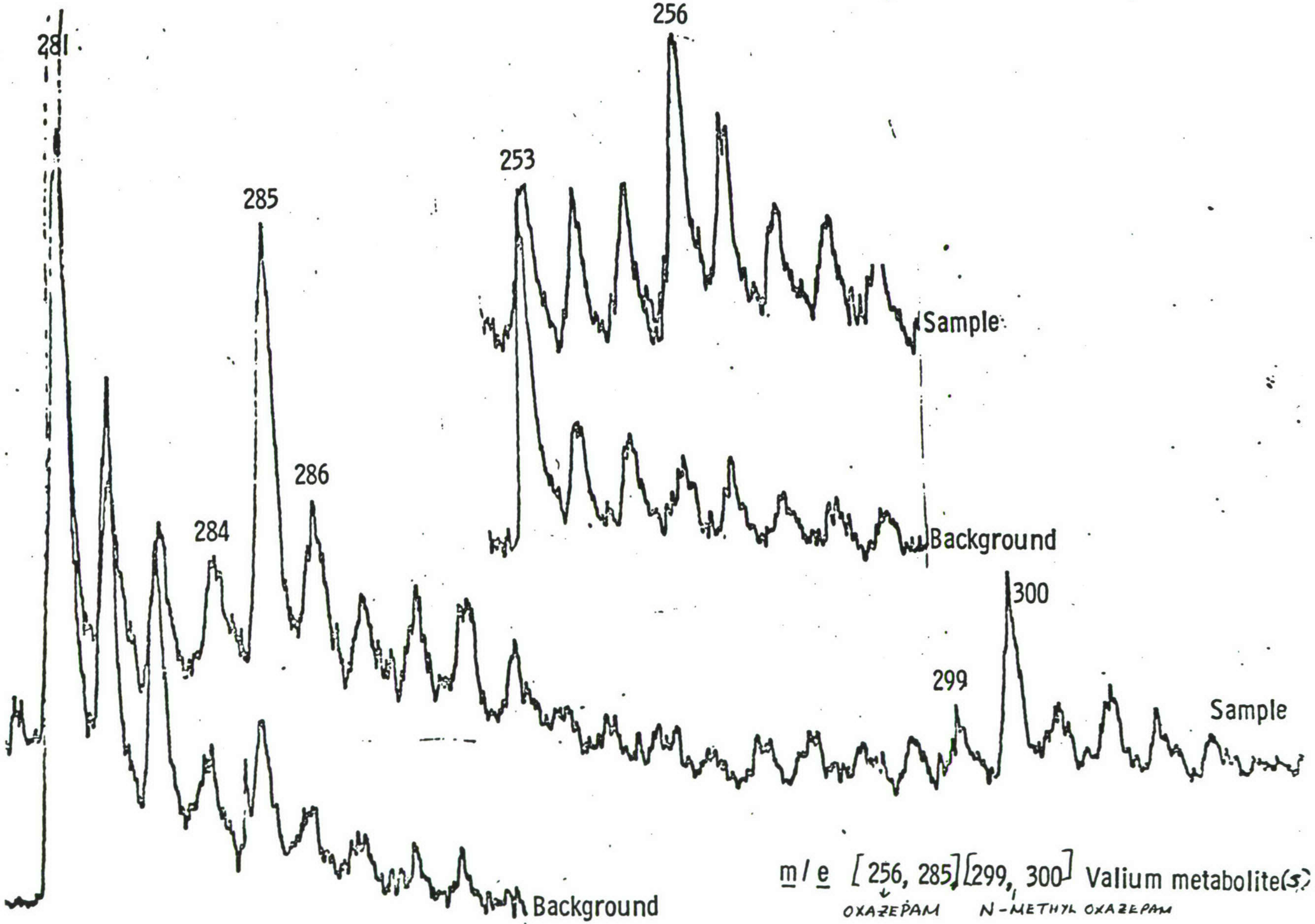


FIGURE 15. ANALYSIS OF DIAZEPAM METABOLITES IN PATIENT (THERAPEUTIC DOSE OF 10mg) URINE

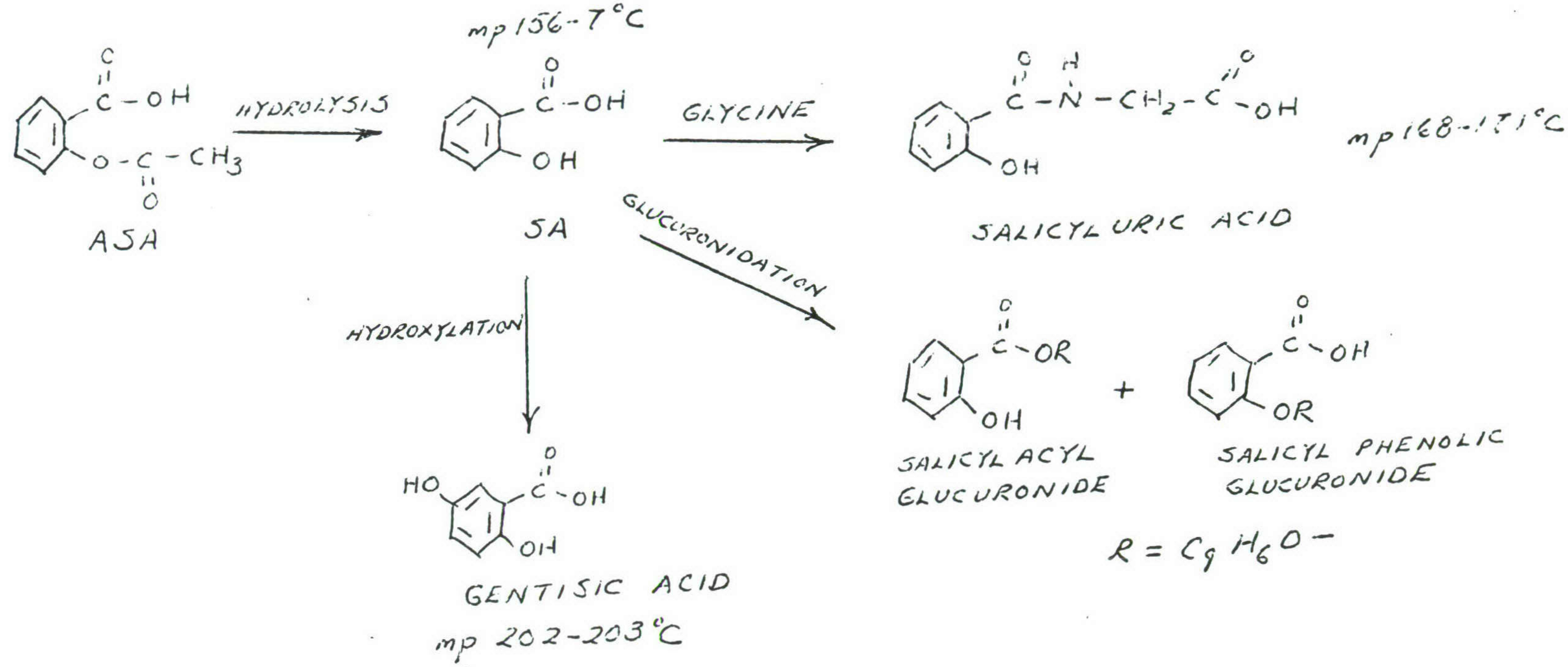


FIGURE 15B METABOLISM OF ASPIRIN

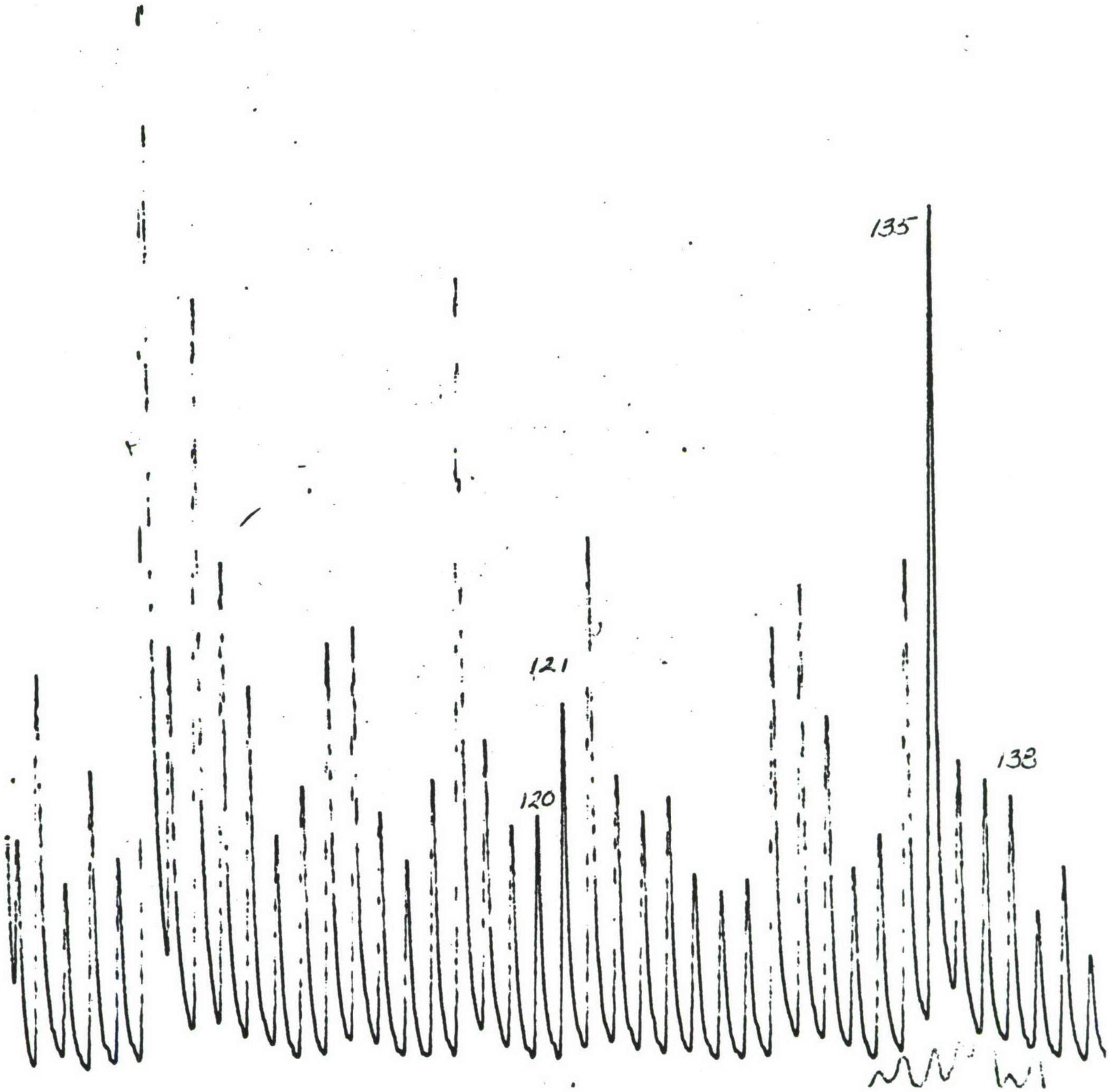


FIGURE 15C CVA SPECTRUM: PATIENT URINE - NO DRUG DOSAGE

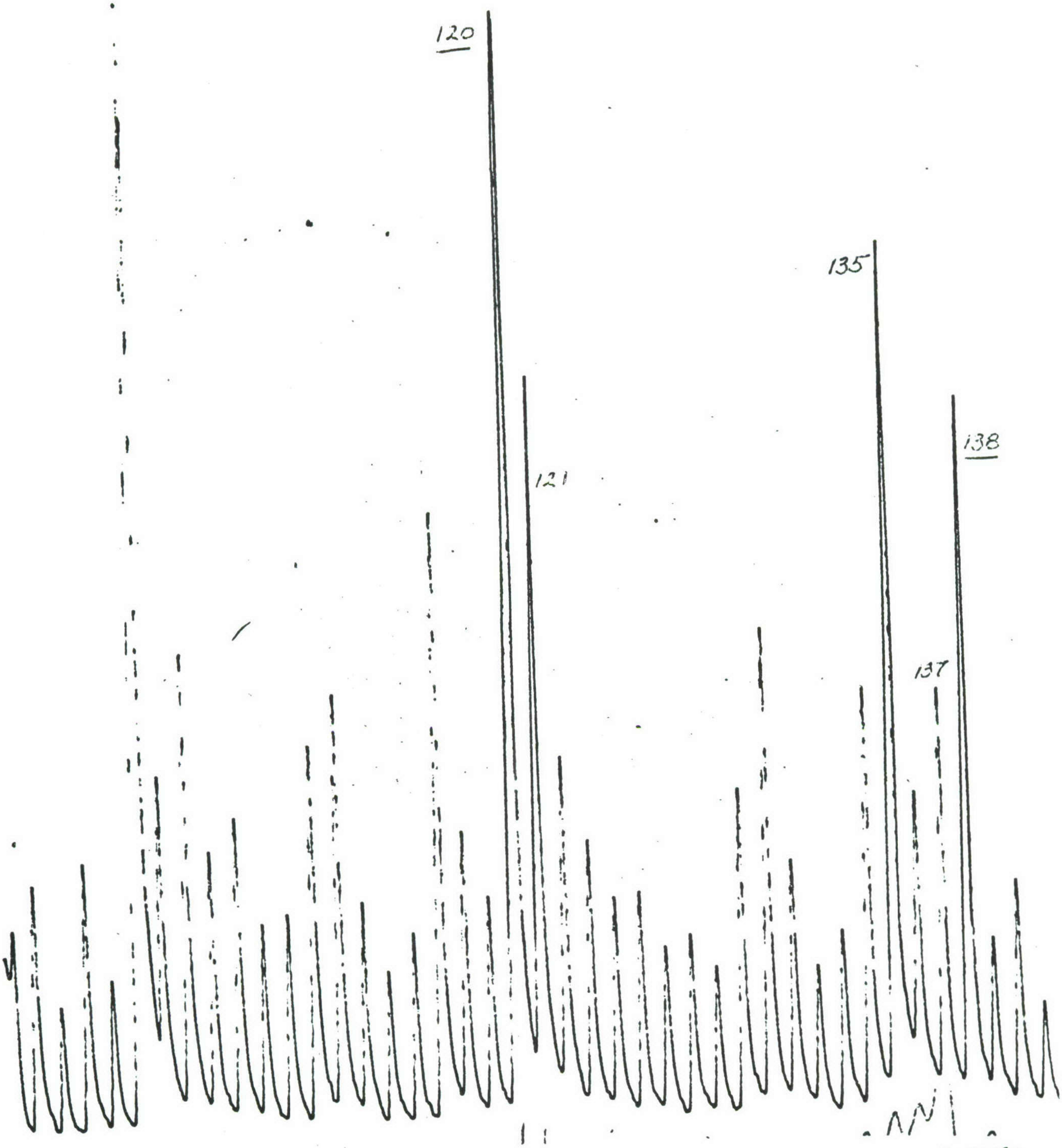


FIGURE 15D CVA SPECTRUM: PATIENT (OF 15C) URINE – 2 HOURS AFTER 600 mg ASA

m/e 120, 138 ⇒ SA

m/e 137 ⇒ SUSPECT SALICYLURIC ACID

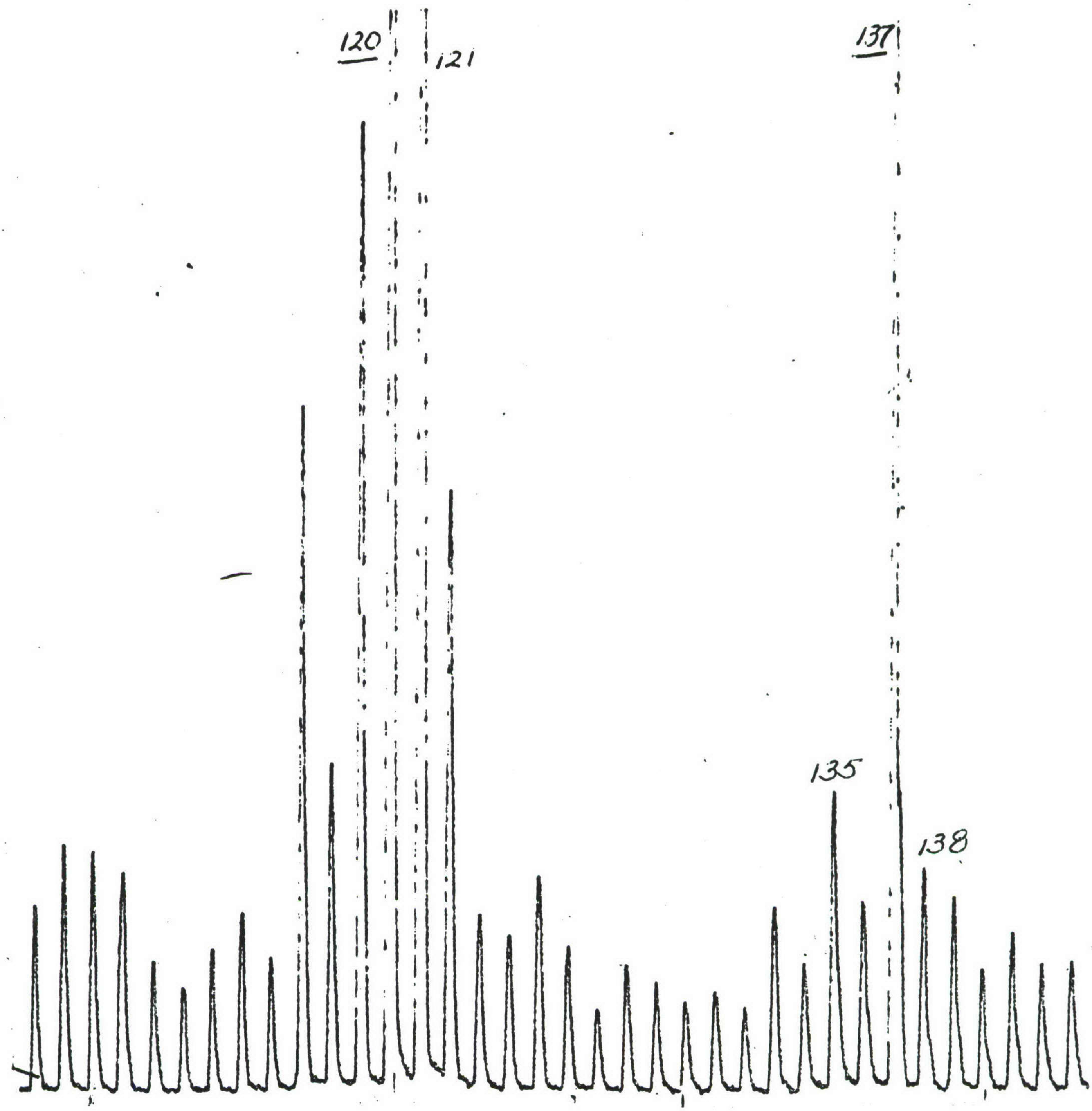


FIGURE 15E CVA SPECTRUM: PATIENT URINE AFTER 600 mg ASA

m/e 137, 120 ⇒ SUSPECT SALICYLURIC ACID

V. Drug Poisoning Analysis

Urinary drug and/or metabolite levels in overdose situations are often similar to those levels in addict urines, where drug abuse is characterized by tolerance development. Thus, heroin addicts maintain a daily habit of 100 mg - 4 g (therapeutic dose of morphine is 10 mg) and secobarbital addicts ingest up to 2 g daily[6] (therapeutic dose 50 mg). Overdose analysis thus constitutes a valid feasibility test for CVA-Mass Spectrometry drug abuse detection.

Rapid overdose analysis is required by physicians in determining whether to employ peritoneal dialysis or hemodialysis, whether to manipulate urine production and acidity so as to enhance drug elimination, whether to send a patient home upon regaining of consciousness, etc.

Drug analysis by CVA-Mass Spectrometry can be performed in either of two modes: MANUAL and COMPUTER. In MANUAL, one scans a specified mass region (i.e., m/e 100-300) at a specified rate (i.e., 100 amu/sec). The problem involved in this mode of operation is the accumulation of unnecessary data. If one desires to screen for a specific drug or drugs, one need only monitor the characteristic ions of each drug of interest. This is achieved by use of the COMPUTER mode.

In the COMPUTER mode, a computer controls the quadrupole mass analyzer, scanning pre-selected peaks rather than the complete mass range. Specifically, if one were interested in screening for morphine and codeine, one would program the system to scan the respective molecular ions at m/e 285, and 299 and perhaps m/e 284, 286, 298, 300 as background peaks in this region. This mode of operation is similar to the technique of mass fragmentography, used in phenothiazine metabolite studies with GC-MS.[7]

For most drugs, several characteristic peaks will be monitored.

For every mass M_i (= mass/charge ratio) to be scanned by the quadrupole mass analyzer the computer has (according to a prior run calibration program) a digital number N_i which is transformed by a D/A converter to a voltage V_{ci}. The analog signal V_{ci} from the D/A converter sets the mass analyzer to sweep through the ion

[6] Information obtained from Dr. Irving Klompers, Haight-Ashbury Free Medical Clinic, SF, CA

[7] C. Sweeley et al, Anal. Chem., 38, 1549 (1966).

peak at M_i in a pre-selected time (typically 1-5 msec) during which the signal area is integrated by an integrating circuit. The integrated signal is fed back to an A/D converter and into the computer which either stores it in the memory (if desired) and/or feeds the signal through a D/A converter to an oscilloscope and/or recorder display.

The initial screening program was identified by the mnemonic SCH symbolizing search, typed on the teletype. The program was to scan for 10 drugs. The program peaks were changed during the development study, and the final form is listed below. Variations of the basic SCH program were labeled SCH-1, SCH-2, and these are described in the appropriate Figures. The SCH program was:

m/e	Screen for
156	pentobarbital
168	secobarbital
178	normal urine background
189	glutethimide and/or α-phenylglutarimide
191	silicone (membrane) background
204	phenobarbital
207	silicone (membrane) background
232	chlorinated phenothiazine
253	oxazepam
266	trifluoromethane - substituted phenothiazine
277	N-demethylated methadone metabolite (2-ethyl-5-methyl-3, 3-diphenyl-1-pyrroline)
281	silicone (membrane) background
282	chlordiazepoxide
283	diazepam
285	morphine

If one or more of these peaks increased significantly, one would then type the mnemonic for an appropriate sub-program which presented additional characteristic ions for the suspected drug. Thus if m/e 168 increased in SCH, one typed in SEC (for secobarbital) and the ions at m/e 167, 168, 195 were presented. The sample intensities could be compared to a standard to confirm the drug identification.

Additional screen programs were added during development for phenothiazines, (PTZ) and narcotics (NAR).

PTZ m/e	
165	urinary background
166	all phenothiazines
167	all phenothiazines
198	all phenothiazines
199	all phenothiazines
210	thioridazine
211	thioridazine
229	thioridazine
230	thioridazine
231	background
232	chlorinated phenothiazine
233	chlorinated phenothiazine
234	chlorinated phenothiazine - M+Z ion
265	silicone (membrane) background
266	trifluoromethane - substituted phenothiazines
267	trifluoromethane - substituted phenothiazines

NAR m/e	
276	N-demethylated methadone metabolite
277	N-demethylated methadone metabolite
278	background
279	background
280	background
281	silicone (membrane) background
282	background or chlordiazepoxide
283	background or diazepam
284	background
285	morphine
286	background
294	methadone
295	silicone (membrane) background
298	background
299	codeine
300	background

Poisonings 1-4 were analyzed at Varian. Samples were obtained from San Francisco General Hospital (SFGH) and refrigerated until analysis. Poisonings 5-14 were analyzed at SFGH on an emergency basis (immediate analysis-answer within 5-10 minutes of sample receipt). Urine samples obtained at SFGH were 20-100 ml. Blood samples were 1-2 ml. Poisonings 1-7 were analyzed with the CVA system in the manual scan mode and 7-14 analyzed with the system in the computer program mode. The recorder print-outs were small and difficult to see, as seen in monthly report 5. Thus, hand-drawn reproductions at 2X real-scale are shown in this report.

All analyses were run under one set of instrument thermal parameters: inlet temperature 275°C, membrane temperature 180°C, analyzer temperature 185°C. The inlet temperature was chosen so as to flash-volatize the least volatile drug species of interest, morphine (melting point 230°C). The membrane temperature was chosen so as to transmit the most polar drug species of interest, again morphine. Less polar drugs are characterized by maximum membrane permeabilities below 180°C but suffer loss relative to peak sensitivity of less than a factor of four.

(1) Phenobarbital (Luminal) "Therapeutic" Dose

Urine and saliva samples were collected from a phenobarbital addict undergoing gradual withdrawal treatment at San Francisco General Hospital.

The withdrawal dose of 700 mg daily would constitute an overdose for a non-addict (100-200 mg is the hypnotic dose). Such a situation exists in other addict-tolerance situations (i.e., secobarbital, morphine). Such cases are thus discussed in this section.

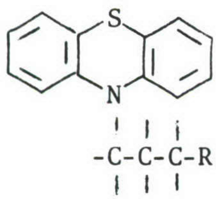
The CVA mass spectra of the urine and a salivary extract are presented in Figs. 16-17. Urinary detection, monitoring the m/e 204 phenobarbital base peak, indicated approximately 20 mg % phenobarbital. Phenobarbital was not detected in the extract of one ml saliva. The phenobarbital detection limit is about 100 ng and thus the salivary drug level is 0.1 μg/ml.

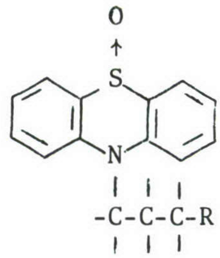
The saliva was worked up by dilution with aqueous pH2 HCl, ultra-filtration through a PM-10 membrane, extraction of filtrate with dichloromethane, concentration of extract to 25 μl and injection into the CVA system.

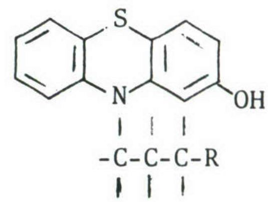
(2) Phenothiazine Poisoning

The patient was admitted into the hospital in a comatose state. Toxicology Lab urine analysis, utilizing both the Forrest reagent test and TLC indicated phenothiazine poisoning. These tests are calss tests and not specific. The patient had a prescription for fluphenazine and thus fluphenazine was suspected.

A urine sample was obtained from SFGH and analyzed by CVA-Mass Spectrometry (Fig. 18). Intense non-background mass peaks were observed at m/e 193, 194, 195, 216, 217, 225, 227, 234, 236, 238, 240, 241, 252, 253, 256, 270. This pattern indicative of a mixture of a phenothiazine having no 2-substituent (ruling out fluphenazine which is 2-CF_3 substituted) and an alkyl chain of ≥ 3 carbons attached to the ring nitrogen, its sulfoxide metabolite(s) and its 2-OH metabolite.

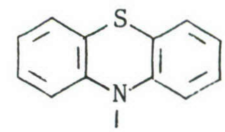




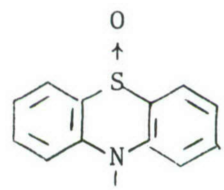


Key individual peak assignments are:

m/e 253 $\quad R\ CH_2\dot{C}HCH_2\overset{+}{C}H_2$, $R\ CH_2\underset{CH_3}{CH}=CH_2$

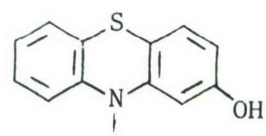
R = 

m/e 252 $\quad R\ CH_2CH=\overset{+}{C}H_2$, $\overset{+}{R}=\underset{CH_3}{CH}CH=CH_2$

or 

m/e 241 $\quad \overset{+}{R}\ (OH)\text{-}CH_2CH_2\cdot$

m/e 240 $\quad R\text{-}CH_2\overset{+}{C}H\text{-}CH_3$

R(OH) = 

m/e 238 $\quad R\text{-}CH_2CH=\overset{+}{C}H$

m/e 236 $\quad \overset{+}{R}=CH\text{-}C\equiv CH$

m/e 227 $\quad \overset{+}{R}\ (OH)\text{-}CH_2\cdot$

m/e 225 $\quad \overset{+\cdot}{R}\ \text{-}CH=CH_2$

The pattern of phenothiazine peaks between m/e 200 - m/e 300 allows one to distinguish non-substituted from -Cl (i.e., chlorpromazine) and $-CF_3$ (i.e., fluphenazine) substituted phenothiazine. Further differentiations requires monitoring the molecular ion region (m/e 280-450) of the various phenothiazines. This problem is discussed in a latter part of this section.

(3) Diazepam (Valium) Poisoning

The patient was admitted into the hospital in a comatose state. The attending physician suspected Valium poisoning on the basis of an empty vial with a Valium prescription on it, found with the patient's effects. A urine sample was submitted for immediate CVA mass spectral analysis.

The urinary pH was adjusted to pH10 and 25 μl injected. A large peak at m/e 253 was observed (Fig. 19) indicating oxazepam (see Section III). Oxazepam is the major urinary metabolite of diazepam[8].

Oxazepam is reported[8] to be excreted in the urine predominantly as the glucuronide conjugate. One ml of urine was hydrolyzed under mild conditions (LM HCl, 30-minute reflux) and the hydrolysate analyzed. An increase in the m/e 253 peak of ~X50 relative to that in unhydrolyzed urine was observed.

(4) Suspected Ethchlorvynol (Placidyl) Poisoning

The patient was admitted to the hospital in a comatose state. The attending physician suspected ethchlorvynol since the patient had been on such a prescription, and the urine had a pungent, aromatic odor suggesting a volatile drug. The physician submitted a sample for CVA mass spectral analysis as a check on his suspicion of ethchlorvynol.

The abundant mass peaks of ethchlorvynol occur below m/e 120, a region subject to interference from normal urinary constituents. However, since ethchlorvynol is a relatively volatile compound, one can reduce the interference by analyzing the head space of warmed (80°C) urine.

The ethchlorvynol ions at m/e 115, 117, 109 were observed. However, one also observed more intense ions at m/e 108, 107 which suggested the presence of another volatile drug or metabolite (Fig. 20).

Common drugs having abundant ions at m/e 108, 107 are phenyrmidol, ethoheptazine and mephenesin. On the basis of volatility, ethoheptazine was suspected.

The patient died before this suspicion could be resolved.

[8] A. F. de Silva and C. V. Puglisi, Anal. Chem. 42, 1725 (1970).

The head-space sampling procedure offers promise for analysis of volatile drugs. Analysis of such drugs is difficult by TLC due to sample evarporation on the chromatoplate.

(5) Diazepam (Valium) Poisoning

The patient was admitted into the hospital in a comatose state. A sample of gastric contents was submitted by the attending physician. An ether extract (no pH adjustment) was obtained and injected (Fig. 21).

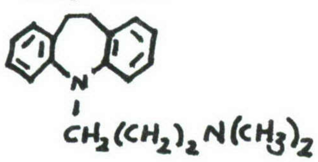
The intense mass peaks at m/e 283, 256, 241, 221 indicate diazepam (see Fig. 4 of Section III). The relatively low intensity peaks at m/e 253, 239, 233 indicate oxazepam, a diazepam metabolite. Toxicology Lab analysis (TLC) indicated diazepam.

(6) Imipramine (Tofranil) Poisoning

The patient was admitted to the hospital on a Sunday, in a comatose state. She died several hours later. TLC analysis was run by the Toxicology Lab on Monday, indicating imipramine and "phenothiazine-like spots." These could be imipramine metabolites or metabolites of a phenothiazine.

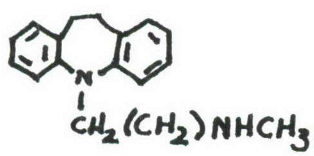
The Toxicology Lab submitted a dried urine extract for CVA Mass Spectrometric analysis. The scan (Fig. 22) had intense peaks at m/e 193-195, 234, 235 suggesting imipramine and/or imipramine metabolites (see Table I). Low intensity peaks at m/e 280, 266 are assigned to the molecular ions of imipramine and desmethylimipramine respectively; that at m/e 251 is assigned to the M-1 ion of didesmethylimipramine.

Imipramine



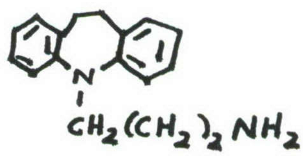
MW 280

Desmethyl metabolite

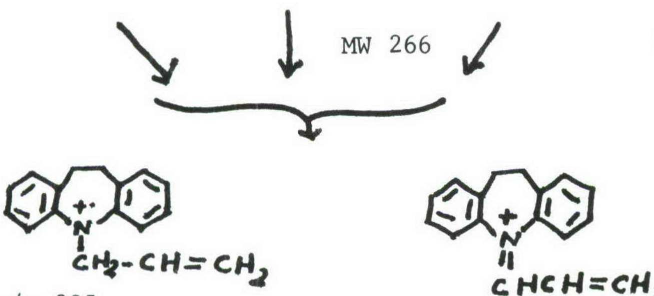


MW 266

Didesmethyl metabolite



MW 252



m/e 235

m/e 234

Fluphenazine has its base peak at m/e 280 but does not yield significant peaks at m/e 193-195, 234, 235. Thus the predominant drug in the urine is imipramine. A relatively low level of fluphenazine could be present however. To resolve this question, one must monitor the strong fluphenazine molecular ion (~40% of M/E 280 base peak[9]) at m/e 437. However, the particular quadrupole unit in the current CVA system does not operate above m/e >350.

The phenothiazine drugs have strong molecular ions at m/e > 350 and thus indicate a need for a CVA system operating up to m/e 450:

phenothiazine	m/e of molecular ion	R.A.
chlorpromazine (Thorazine)	318	10%
flupromazine (Vesprin)	352	8
prochlorperazine (Compazine)	373	37
trifluoperazine (Stelazine)	407	18
acetophenazine (Tindal)	411	21
perphenazine (Trilafon)	403	10
fluphenazine (Prolixon)	437	42
thioridazine (Mellaril)	370	35

(7) Chlorinated Phenothiazine Poisoning

The patient was admitted to the hospital in a comatose condition. Phenothiazine poisoning was suspected in that the patient was undergoing psychiatric chemotherapy on trifluoperazine and chlorpromazine.

The CVA-Mass Spectrometer was set in the SCH computer program mode, the urine adjusted to pH 10 and injected (10 µl). The observed increase in m/e 232 (Fig. 23A) suggested a chlorinated phenothiazine. The system was switched to the phenothiazine PTZ program mode. Intense peaks at m/e 198, 199, 232, 233 indicate chlorinated phenothiazine, (Fig. 23B).

Commercially available chlorinated phenothiazines are chlorpromazine, prochlorperazine and perphenazine. Standards of these components indicate similar peak patterns in the PTZ program (Fig. 24). Phenothiazines are predominantly metabolized in urine as sulfoxide, hydroxyl and demethylated derivatives and exact matching of urinary peaks to unmetabolized drug is not recommended.

[9] J. T. Gilbert and B. J. Millard, Org. Mass Spec., 2, 17 (1969).

Specification of the particular chlorinated phenothiazine requires monitoring of the molecular ion, M[the sulfoxide metabolite gives a strong M-16 peak (loss of oxygen) coinciding with the M peak of unchanged drug] at m/e 318, 373, 403 for chlorpromazine, prochlorperazine, perphenazine respectively.

Absence of significant intensity at m/e 266, 267 and m/e 229, 230 eliminates trifluoperazine and thioridazine as the poisoning drug(s). The patient died two days later. Since chlorpromazine is a relatively non-toxic drug, prochlorperazine and perphenazine are suspected.

The Toxicology Lab analysis (TLC) indicated phenothiazine.

Similar drug levels were observed on days 2 and 3 post-overdose. Phenothiazines are known to exhibit high tissue uptake and slow excretion.

(8) Chlorinated Phenothiazine Poisoning

The patient, a two-year old female, was admitted to the hospital in a comatose condition. The patient had constricted pupils, arousing suspicion of a heroin poisoning. Naloxone (Narcan) was administered, but a positive response was not elicited.

A Toxicology Lab screen indicated phenothiazine in a urine sample. The child's mother was on prochlorperazine chemotherapy and the physician was curious as to whether the poisoning drug was a chlorinated phenothiazine (i.e., did the mother administer the drug). A urine sample was submitted for analysis.

The urine was adjusted to pH 10 and injected (10 μl) with the system set at program PTZ-1. The intense ions at m/e 232, 233 indicate chlorinated phenothiazine (Fig. 25).

(9) Glutethimide (Doriden) Poisoning

The patient was admitted to the hospital in a comatose condition. A urine sample was adjusted to pH 2 and injected (10 μl) with the system set at SCH-1. The intense m/e 189 ion (Fig. 26) suggested α-phenylglutarimide (urinary metabolite of glutethimide). Another sample was injected with the system switched to DOR. The intense ions at m/e 146, 161, 189 indicated α-phenylglutarimide, and thus a glutethimide poisoning.

Toxicology Lab analysis (TLC) confirmed this result.

(10) Secobarbital (Seconal) Poisoning

The patient was admitted to the hospital in a comatose state. A sample of gastric aspirate was submitted for emergency analysis. The gastric contents were pink-red. Ten ul of a 1:3 chloroform extract of the sample were injected with the system set at SCH-2 program. An intense peak at m/e 168 suggested secobarbital (Fig. 27). The system was switched to SEC and the intense m/e 167, 168 doublet and m/e 195 ion confirmed secobarbital. The color of the gastric contents was thus due to the red secobarbital capsule.

(11) Phenobarbital (Luminal) Poisoning

The patient was admitted to the hospital in a comatose state. Urine and blood samples were submitted. A 10 µl acidic extract (chloroform, 3:1) was injected with the system set at SCH-3. The intense m/e 204 ion suggested phenobarbital (Fig. 28A). The system was switched to PHE and another sample injected. The intense m/e 146, 204, 232 ions (compare to the standard scan, 28B vs 28C) confirmed phenobarbital.

The attending physician requested a blood barbiturate level. A 10 µl acidic extract (chloroform, 1:1) of serum was analyzed to contain approximately 4 mg % phenobarbital. The patient's clinical symptoms (comatose, arflexive) usually indicate a blood level of 5-10 mg%.[10]

(12) Heroin or Morphine Poisoning

The patient was admitted to the hospital in a comatose state. Toxicology Lab urine analysis (TLC) had indicated morphine, and thus either a heroin or morphine overdose. Mr. Udo Börner (toxicologist) submitted a sample of the hydrolyzed urine to confirm his morphine finding and check for the presence of codeine.

The extract was dissolved in acetone and a 10 µl (1/50 of sample) aliquot injected with the system set at NAR. The strong ion at m/e 285 (Fig. 29) confirms morphine at a level of ~2 ug/10 ul or 2 X 50 = 100 µg from 10 ml urine, or 1 mg % morphine. This would not be a high level for an addict heroin dose and suggests either that the poisoned patient was a new user on a low dose or that the patient was admitted to the hospital 12 hours or more after overdose.

Codeine was not detected, agreeing with Mr. Börner's TLC finding.

[10] I. Sunshine and E. Hackett, Am. J. Clin. Path. 24, 1133 (1954).

(13) Secobarbital (Seconal) Poisoning

The patient was admitted to the hospital in a comatose state. His admission situation is described below:

Tues., May 16, 1972 ★★★ San Francisco Chronicle 7

A Mysterious Death In S.F. Jail Cell

A 24-year-old man was found dead in his cell at Taraval Station yesterday morning, police reported, and his companion was rushed to Mission Emergency Hospital suffering from a mysterious ailment.

The dead man was identified as Shelton Ng, a handyman of 1318 Funston avenue. Police said he and James Chan, 23, a student of 1024 Jackson street, were picked up and put in "protective custody" when they were seen reeling on the sidewalk at Funston and Irving street about 1:15 a.m. yesterday.

Police said they assumed the two were drunk, and planned to release them without charge in the morning.

When officers discovered Ng dead at 7:35 a.m. yesterday, they turned to Chan's adjacent cell and found that he was incoherent.

Chan, who was in serious condition yesterday, told officers that he and Ng had been jumped and beaten without provocation Saturday night by an unidentified man in Berkeley.

Officers said Chan was being tested, and an autopsy was being performed on Ng's body, to see what they had eaten or drunk recently.

Homicide inspector Ken Manley said Ng's body showed no sign of a beating.

"We're trying to determine what happened," Manley said.

The attending physician at MEH submitted a urine sample to determine the patient's "mysterious ailment".

An acidic ether extract of the urine (1:1) was injected with the system set at SCH. The intense off-scale peak at m/e 168 suggested secobarbital. The system was switched to SEC and the intense m/e 167, 168 doublet and m/e 195 ion confirmed secobarbital (Fig. 30).

The moderately intense m/e 156, 189 ions suggest the possibility of pentobarbital and α-phenylglutarimide. However, visual inspection of the mass display with the system switched to MANUAL, m/e 140-200 indicate insufficient m/e 141 intensity to warrant pentobarbital and insufficient m/e 146, 161 to warrant α-phenylglutarimide.

The toxicology Lab analysis confirmed the secobarbital finding.

(14) Phencyclidine Poisoning

The patient was admitted to the hospital in a comatose state. The Toxicology Lab was closed (Sunday) and a private lab gastric analysis indicated phencyclidine (PCP).

The intensive Care Unit physician requested a confirmatory analysis on an admission urine. The literature (see Table I) lists strong ions at m/e 200, 242, 243 for PCP. A sample of PCP was procured and a manual scan indicated the aforementioned ions and an ion at m/e 186.

A PCP program was entered into the computer and the basic extract of the urine and a standard were run (Fig. 31). The intense m/e 200, 242, 243 ions confirmed the PCP identification.

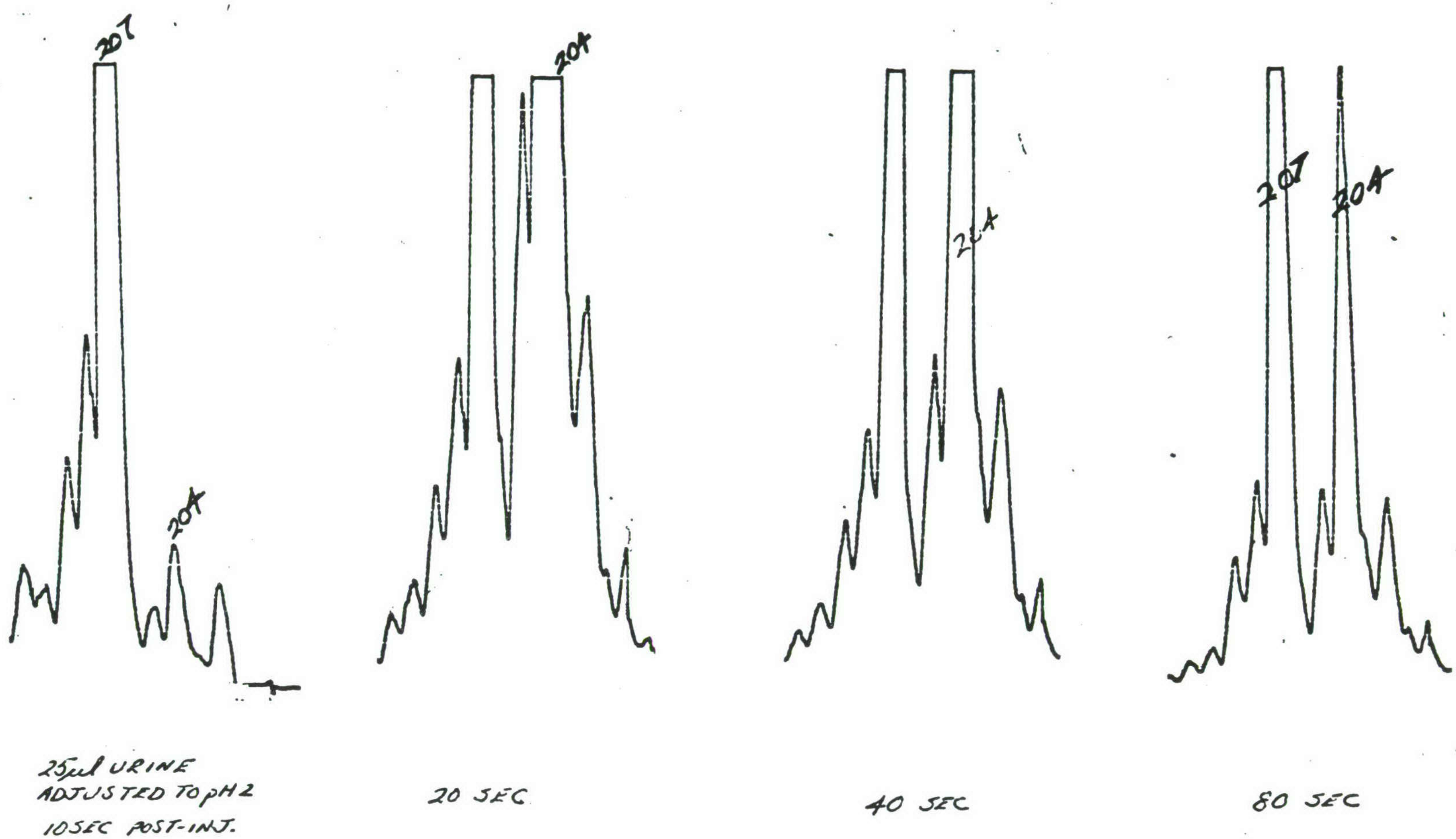
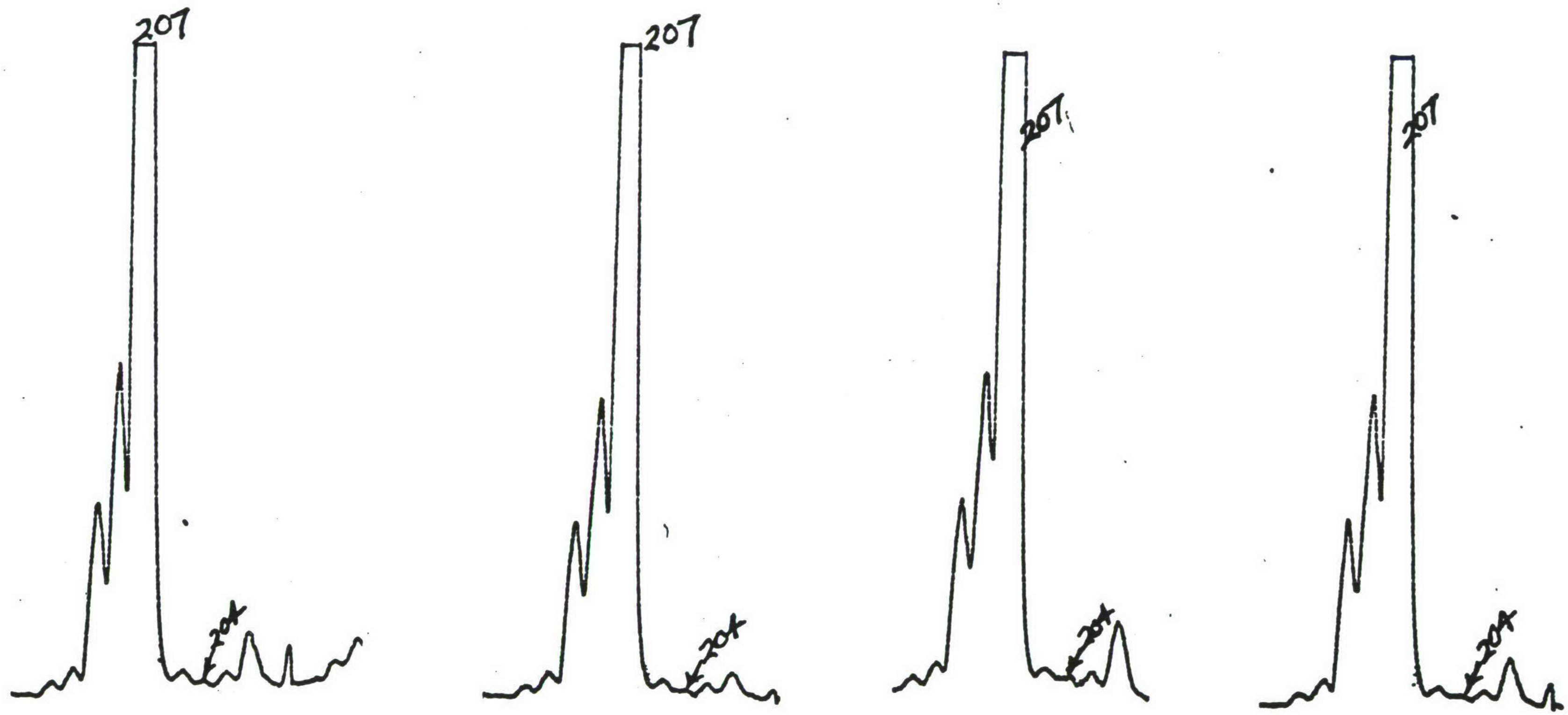


FIGURE 16
CVA MASS SPECTRUM
25μl URINE
m/e 204 ⇒ PHENOBARBITAL



25 µl EXTRACT OF
1 ml SALIVA
10 SEC POST-INJ.

20 SEC

40 SEC

80 SEC

FIGURE 17
CVA MASS SPECTRUM
25 µl EXTRACT OF 1 ml SALIVA

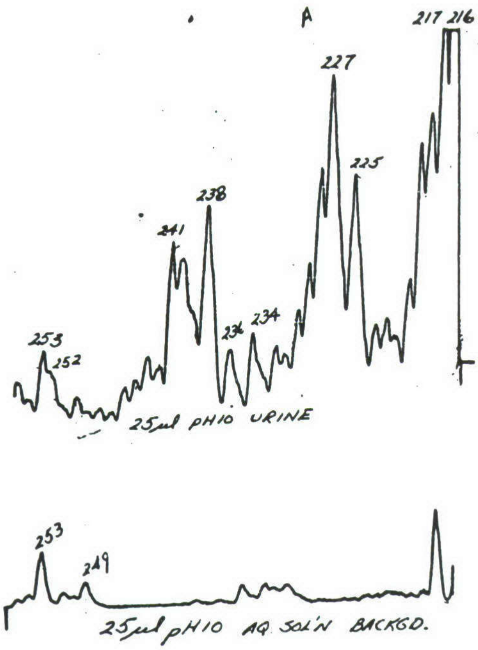


FIGURE 18A 25µl URINE ADJUSTED TO pH10
m/e 215-255

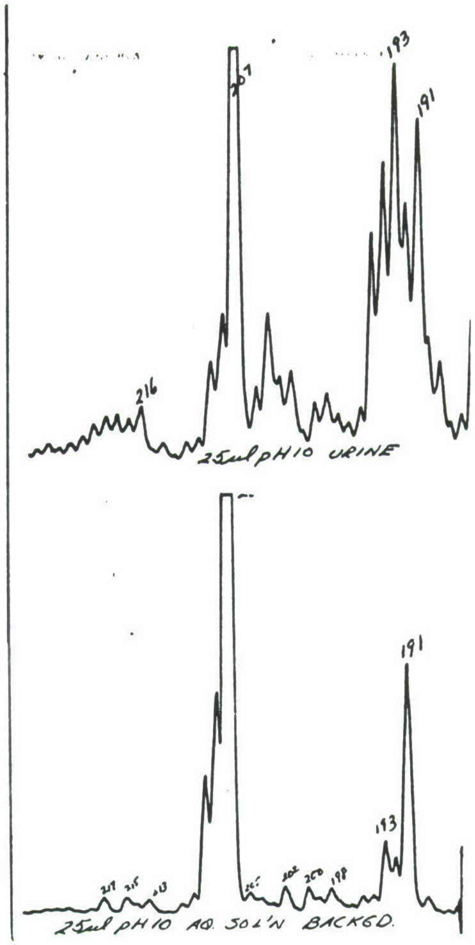


FIGURE 18B
25µl URINE, pH10
m/e 187-226

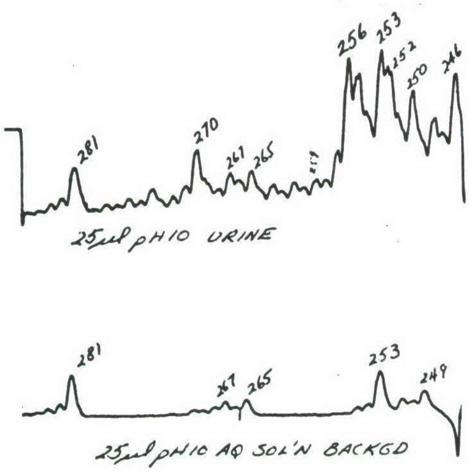


FIGURE 18C 25µl URINE, pH10
m/e 246-285

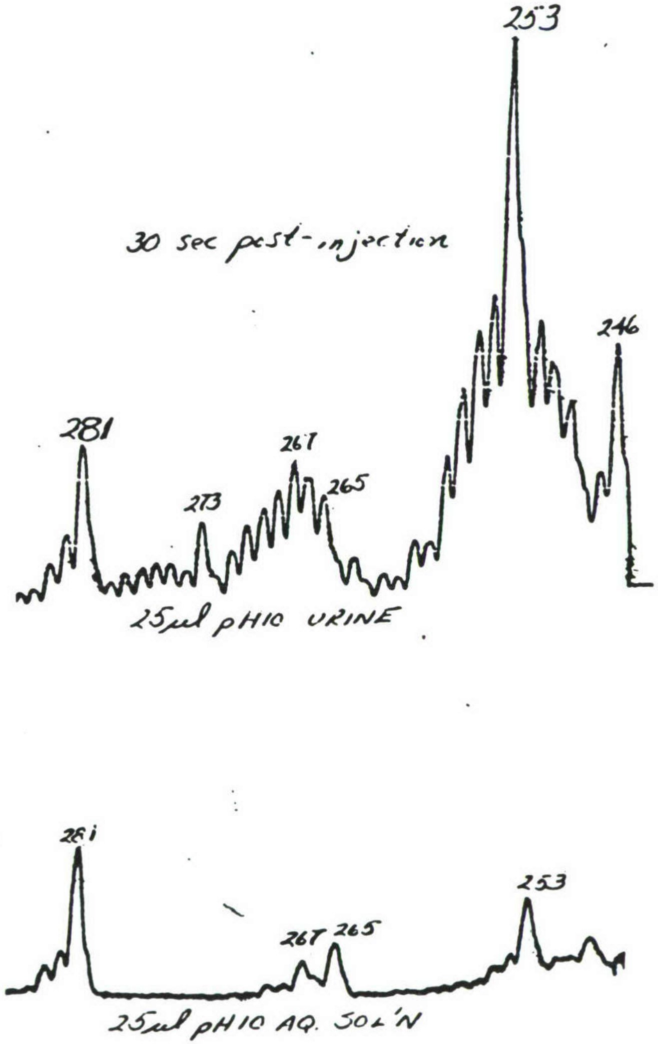


FIGURE 19A 25µl UNHYDROLYZED URINE

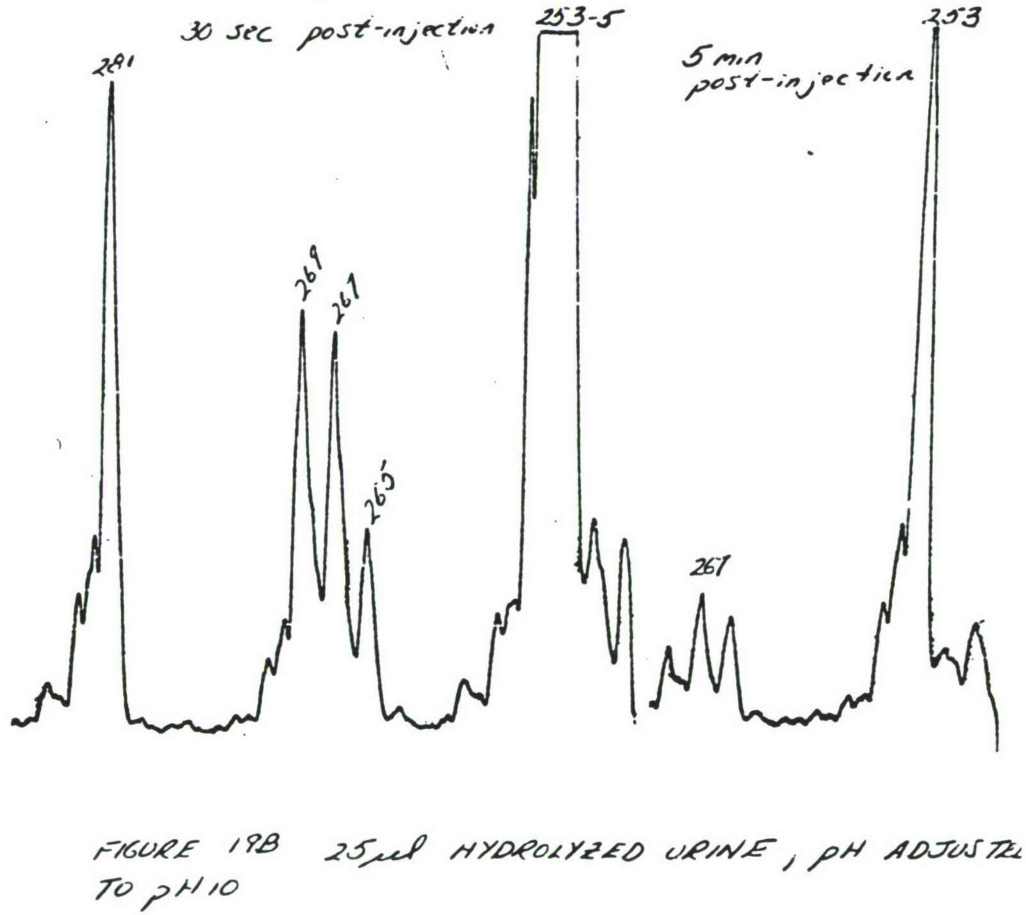


FIGURE 19B 25µl HYDROLYZED URINE, pH ADJUSTED TO pH10

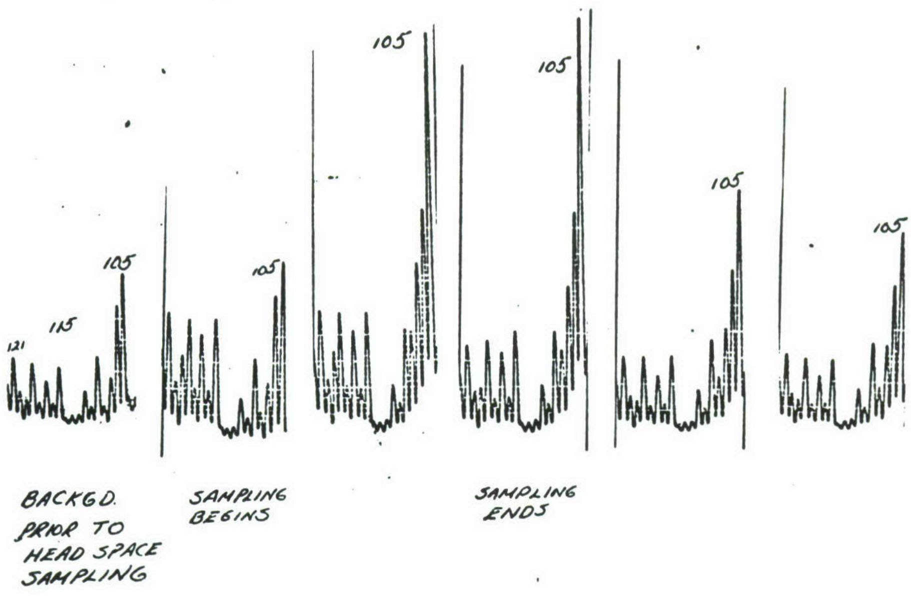


FIGURE 20A HEAD SPACE SAMPLING OF "NORMAL" URINE, WARMED AT 80°C
m/e 104-121 SCANNED EVERY ~3 SEC
m/e 105 ⇒ C_6H_5-C≡O⁺ CHARACTERISTIC OF BENZOIC ACID SPECIES

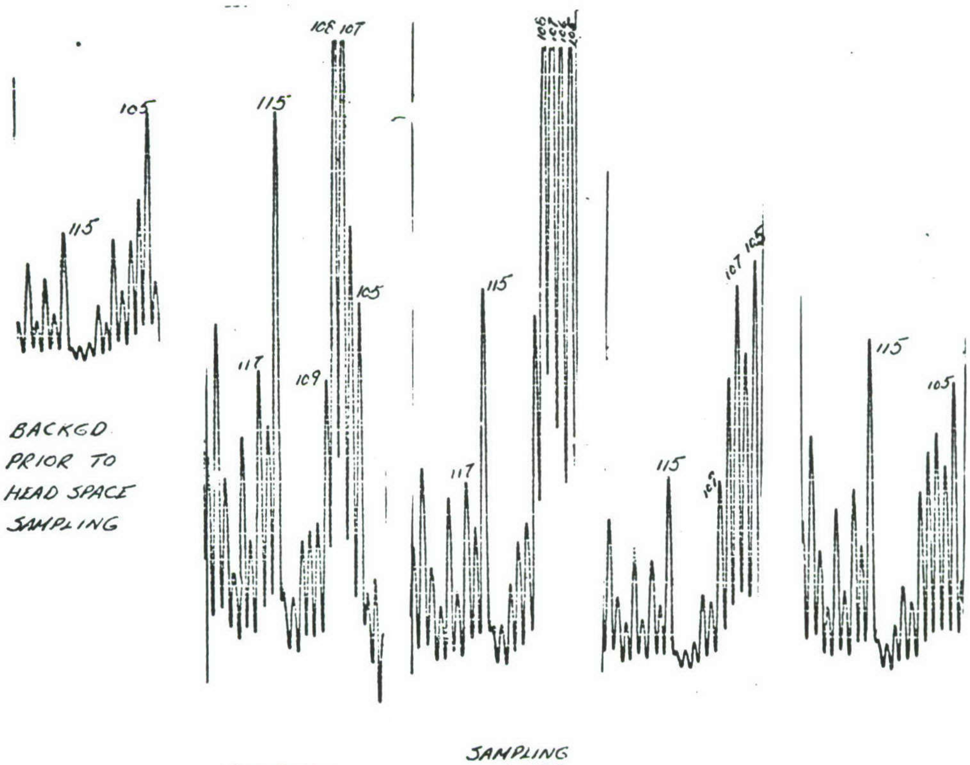


FIGURE 20B HEAD SPACE SAMPLING OF SUSPECTED ETHCHLORVYNOL OVERDOSE URINE

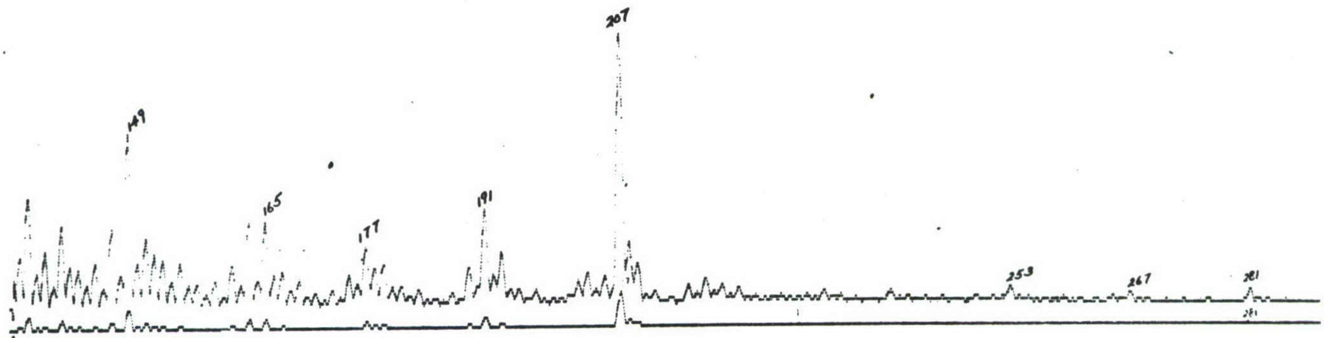


FIGURE 21A 1x10^{-9} GAIN
10µl ETHER BLANK

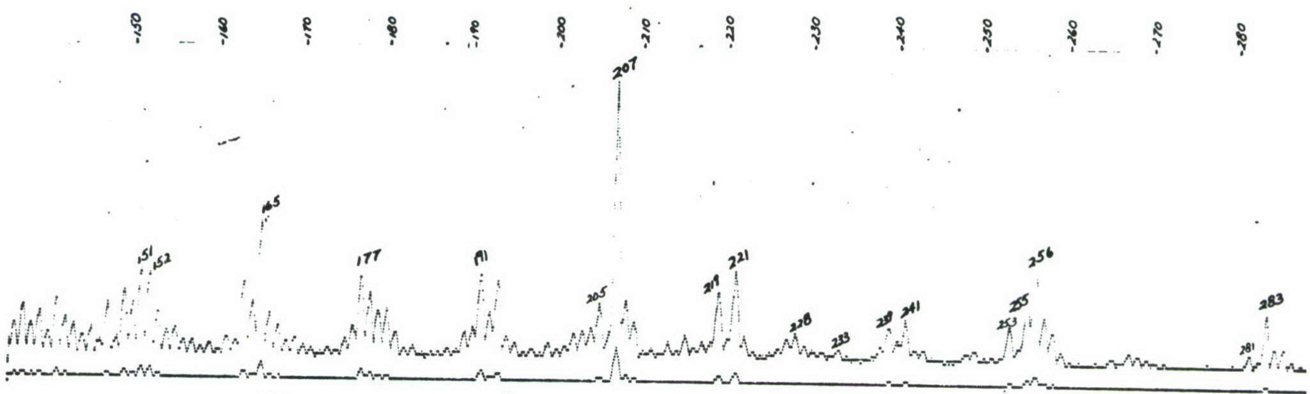


FIGURE 21B 1x10^{-9} GAIN
10µl of 5ml ETHER EXTRACT 15 sec post-injection
OF ~5ml GASTRIC CONTENTS
(REPRESENTS 10µl G.CONTENTS)
-OVERDOSE

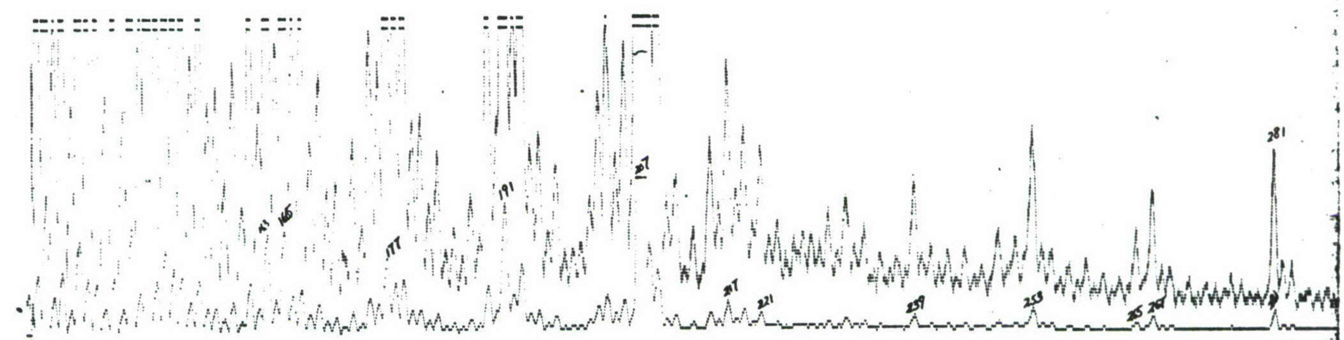


FIGURE 21C 1x10^{-10} GAIN
10µl ETHER BLANK

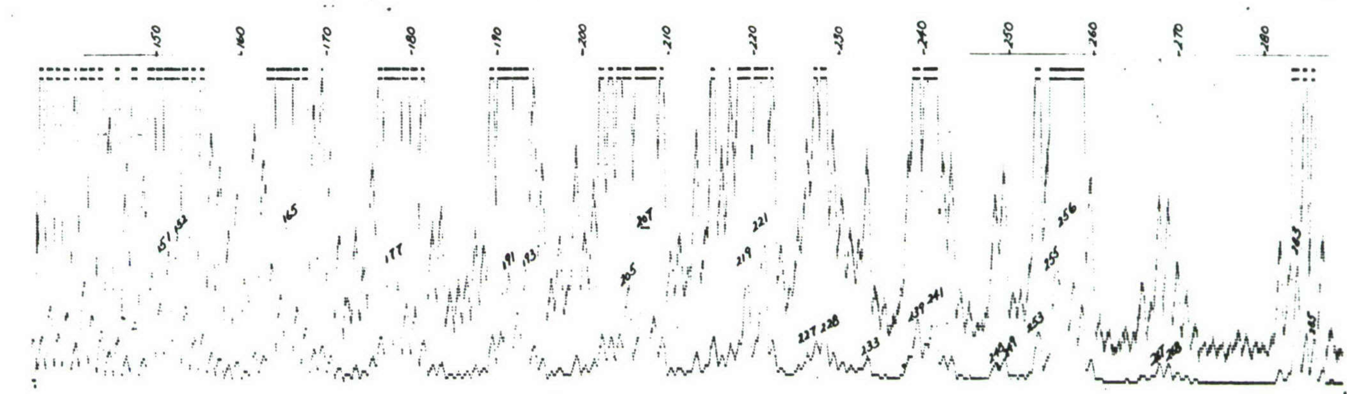


FIGURE 21D 1x10^{-10} GAIN
10µl of 5ml ETHER EXTRACT 18 SEC POST-INJ
OF ~5ml GASTRIC CONTENTS
-OVERDOSE

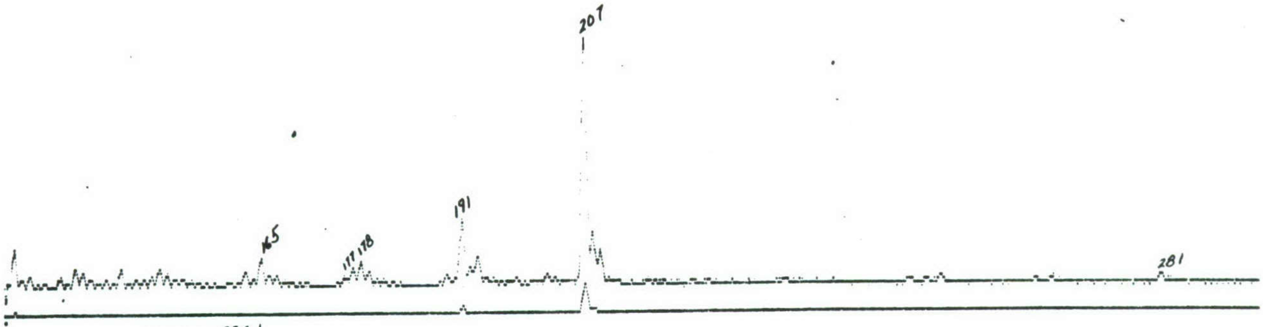


FIGURE 22A
10µl ACETONE BLANK

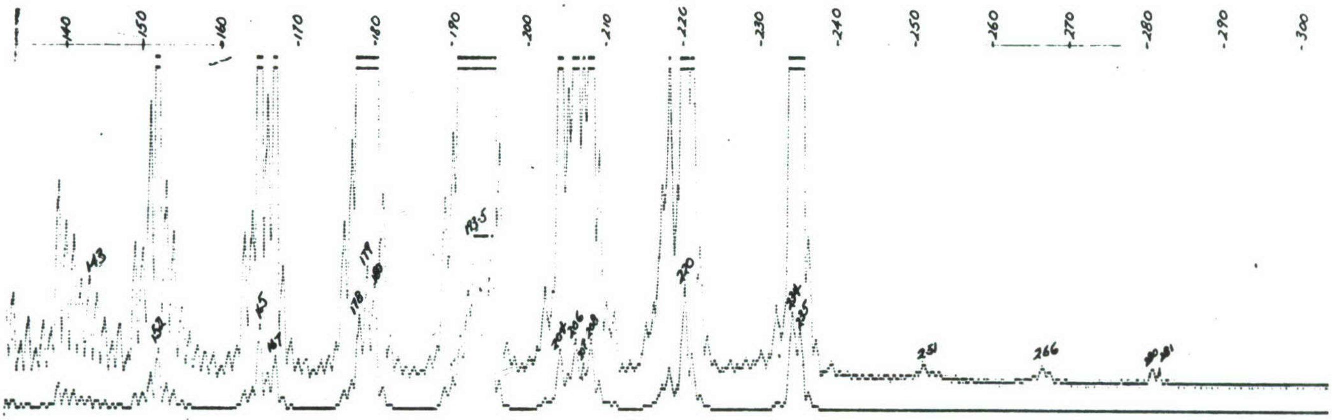


FIGURE 22B
10µl OF 20ml CHLOROFORM EXTRACT
OF 20ml URINE, pH 9
EXTRACT DRIED, REDISSOLVED IN ACETONE
-OVERDOSE (DEATH)

1×10^{-9} GAIN
15 sec post-injection

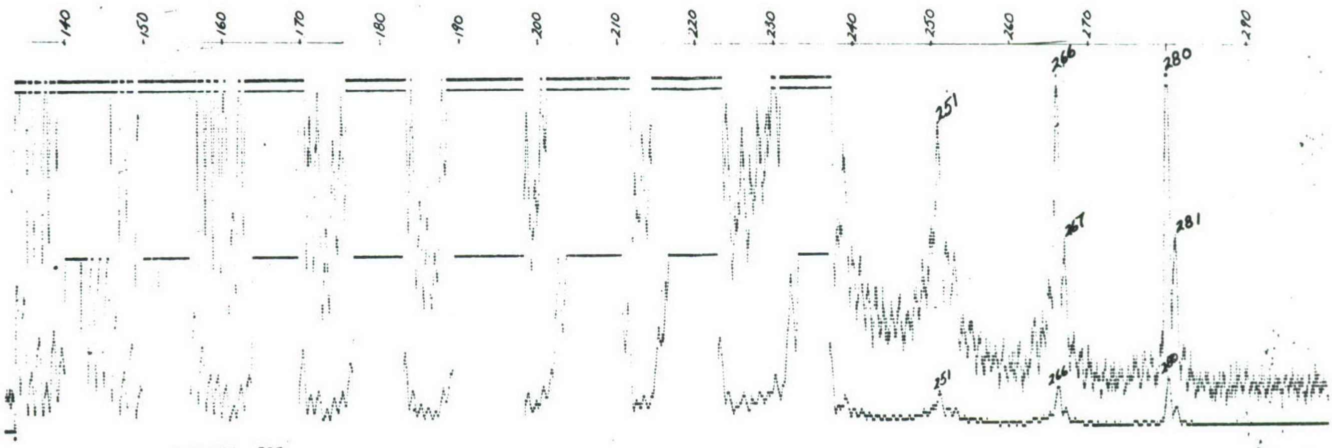


FIGURE 22C
10µl of 20ml CHLOROFORM EXTRACT
OF 20ml URINE, pH 9
EXTRACT DRIED, REDISSOLVED IN ACETONE
-OVERDOSE (DEATH)

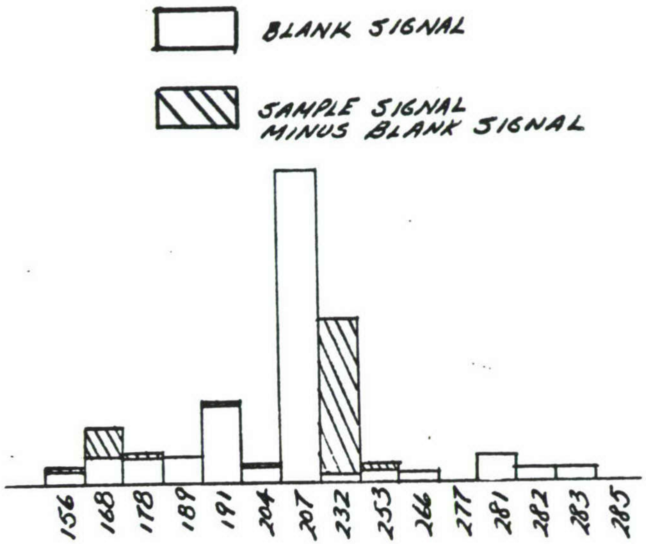


FIG. 23A 10 μl OVERDOSE URINE
ADJUSTED TO pH 10
SCH PROGRAM

SCH

INTENSITY, ARBITRARY UNITS

m/e	BLANK	SAMPLE	S/B	S-B
156	3	4		1
168	6	12	2.0	6
178	5	7	1.4	2
189	5	5	1.0	0
191	16	17	1.1	1
204	3	4	1.3	1
207	64	64	1.0	0
232	2	34	17.0	32
253	3	4	1.3	1
266	2	2	1.0	0
277	0	0	1.0	0
281	5	5	1.0	0
282	3	3	1.0	0
283	3	3	1.0	0
285	0	0	1.0	0

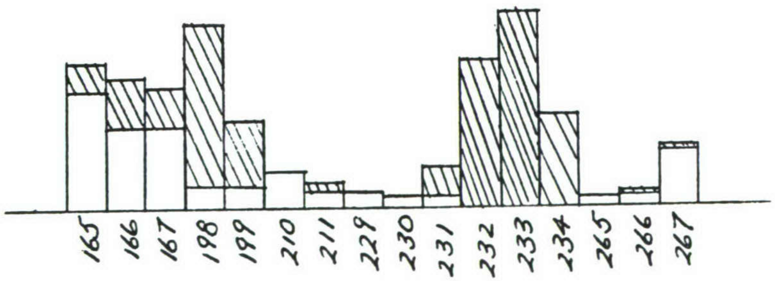
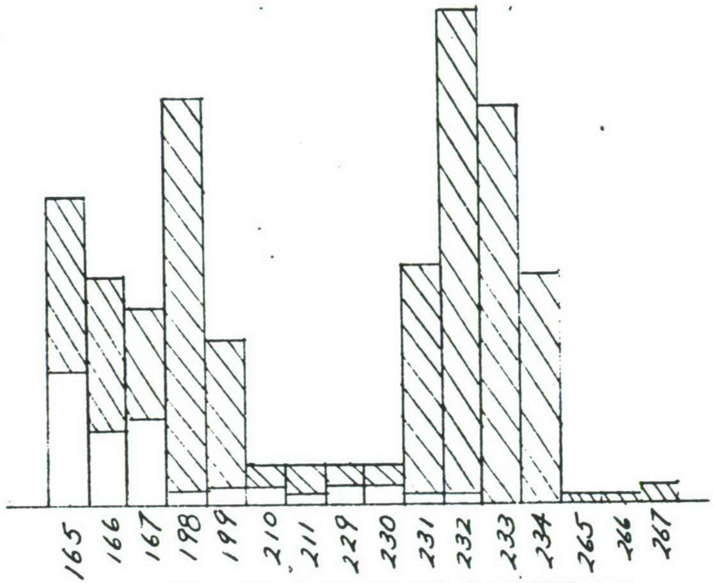


FIGURE. 23B 10 μl OVERDOSE URINE
ADJUSTED TO pH 10
PTZ PROGRAM

PTZ

m/e	INTENSITY S-B	R.A.
165	6	15
166	11	28
167	8	20
198	33	83
199	14	35
210	0	0
211	2	5
229	0	0
230	0	0
231	6	15
232	30	75
233	40	100
234	18	45
265	0	0
266	1	3
267	2	5



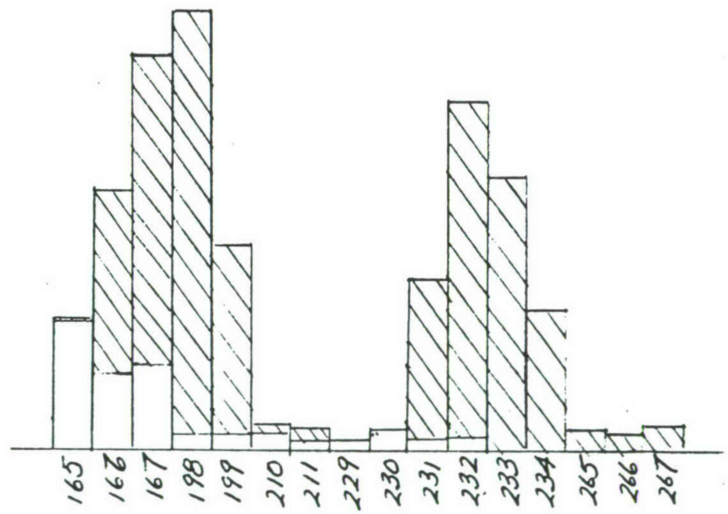
BLANK SIGNAL

SAMPLE SIGNAL MINUS BLANK SIGNAL

PTZ

m/e	S-B	R.A.
165	36	37
166	30	31
167	23	23
198	79	81
199	30	31
210	4	4
211	6	6
229	4	4
230	4	4
231	46	47
232	98	100
233	80	82
234	46	47
265	2	2
266	2	2
267	4	4

FIGURE 24A 10μl URINE OF PATIENT ON CHLORPROMAZINE CHEMOTHERAPY (PTZ)



PTZ

m/e	S-B	R.A.
165	1	1
166	37	43
167	63	73
198	86	100
199	38	44
210	2	2
211	3	4
229	0	0
230	0	0
231	33	38
232	69	80
234	56	65
265	5	6
266	4	5
267	6	7
233	55	64

FIGURE 24B AQ. pH10 SOL'N OF PROCHLORPERAZINE (PTZ)

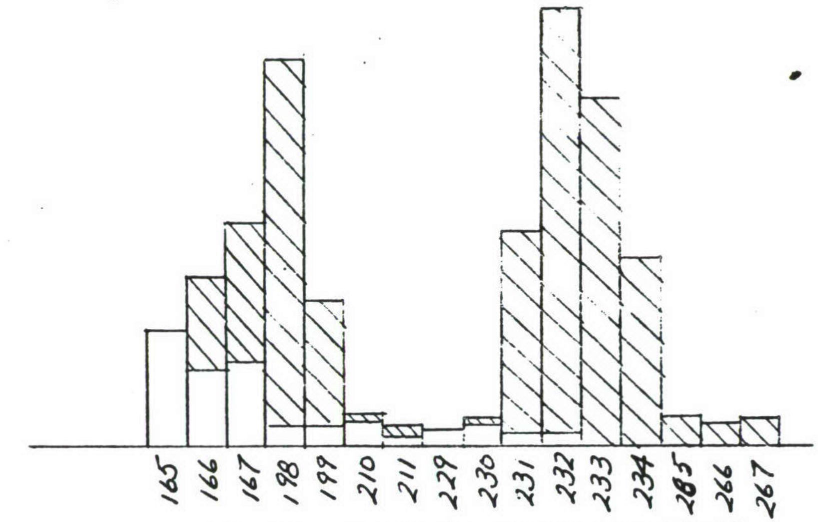
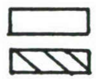


FIGURE 24C AQ. pH10 SOL'N OF PERPHENAZINE (PTZ)

PTZ		
m/e	S - B	RA
165	0	0
166	19	22
167	28	33
198	75	87
199	25	29
210	2	2
211	2	2
229	0	0
230	2	2
231	41	48
232	86	100
233	39	45
234	36	42
265	6	7
266	5	6
267	6	7



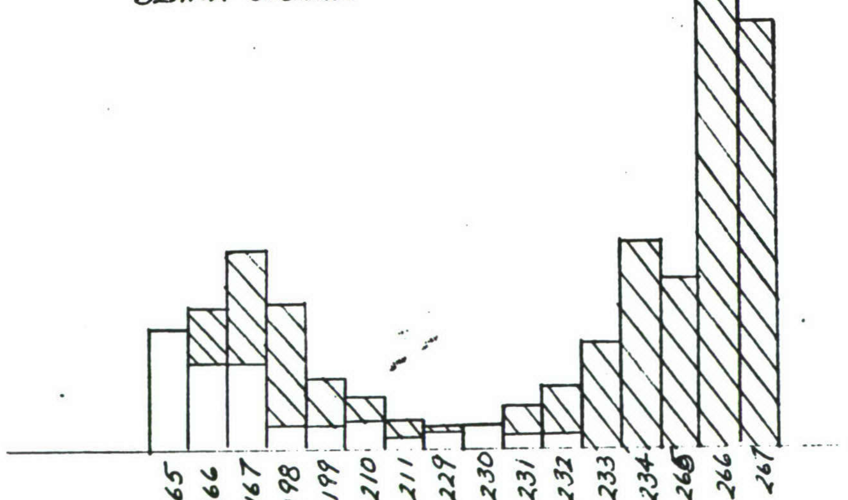


FIGURE 24D AQ. pH10 SOL'N OF TRIFLUOPERAZINE (PTZ)

PTZ		
m/e	S-B	RA
165	0	0
166	11	11
167	23	23
198	25	26
199	9	9
210	5	5
211	4	4
229	1	1
230	0	0
231	6	6
232	10	10
233	22	22
234	42	43
265	34	35
266	98	100
267	88	89

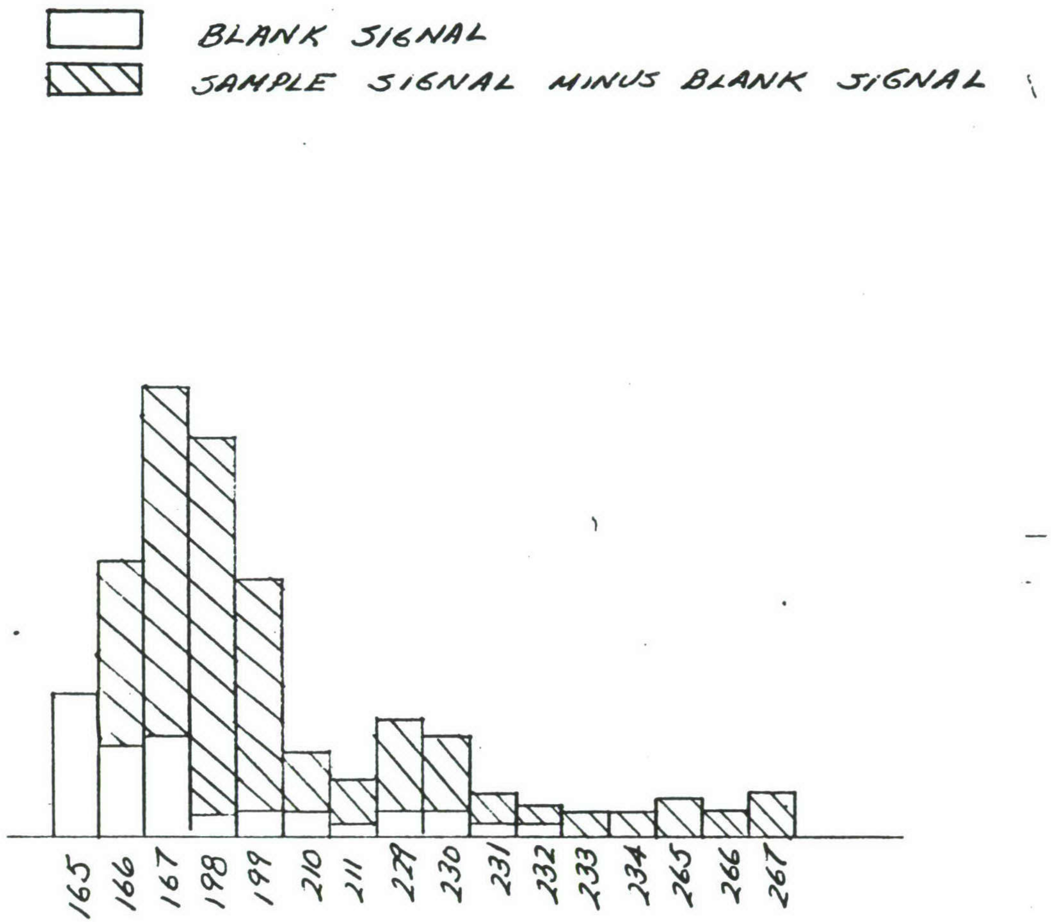


FIGURE 24E AQ. pH10 SOL'N OF THIORIDAZINE
(VIZ)

	PTZ	
m/e	S-B	RA
165	0	0
166	32	49
167	61	93
198	66	100
199	40	61
210	10	15
211	7	11
229	16	24
230	13	20
231	5	8
232	3	5
233	4	6
234	4	6
265	7	11
266	4	6
267	7	11

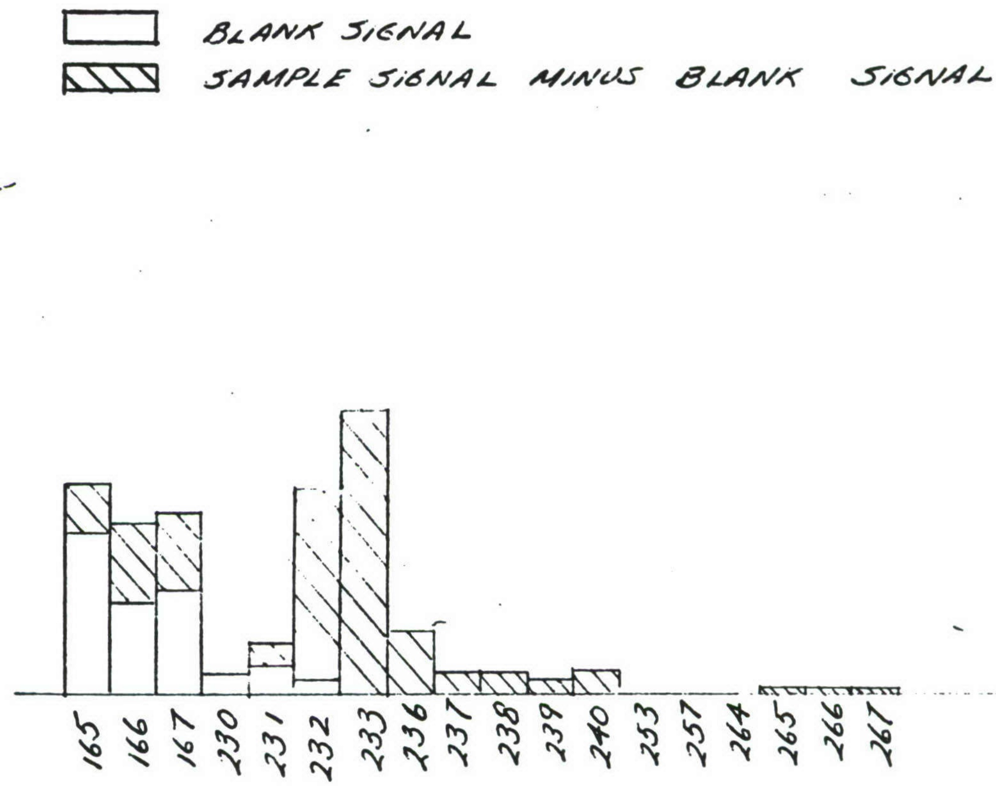


FIGURE 25 10µl OVERDOSE URINE

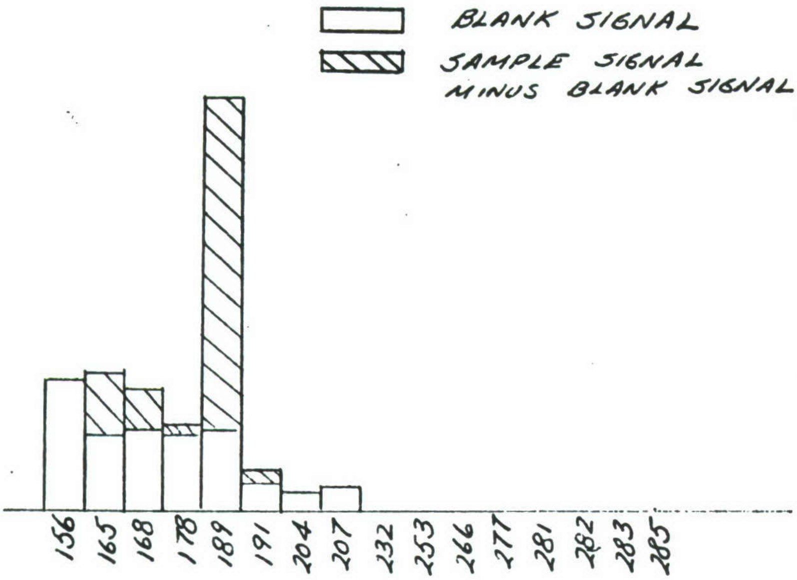


FIGURE 26A 10µl OVERDOSE URINE
ADJUSTED TO pH2
SCH-1

m/e	S/B	S-B
156	0	0
165	1.8	13
168	1.5	8
178	1.1	2
189	4.2	66
191	1.6	3
204	0	0
207	0	0
	↓	↓

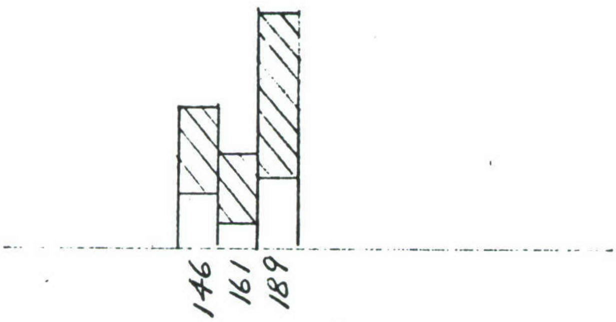


FIGURE 26B 10µl OVERDOSE URINE
ADJUSTED TO pH2
DOR

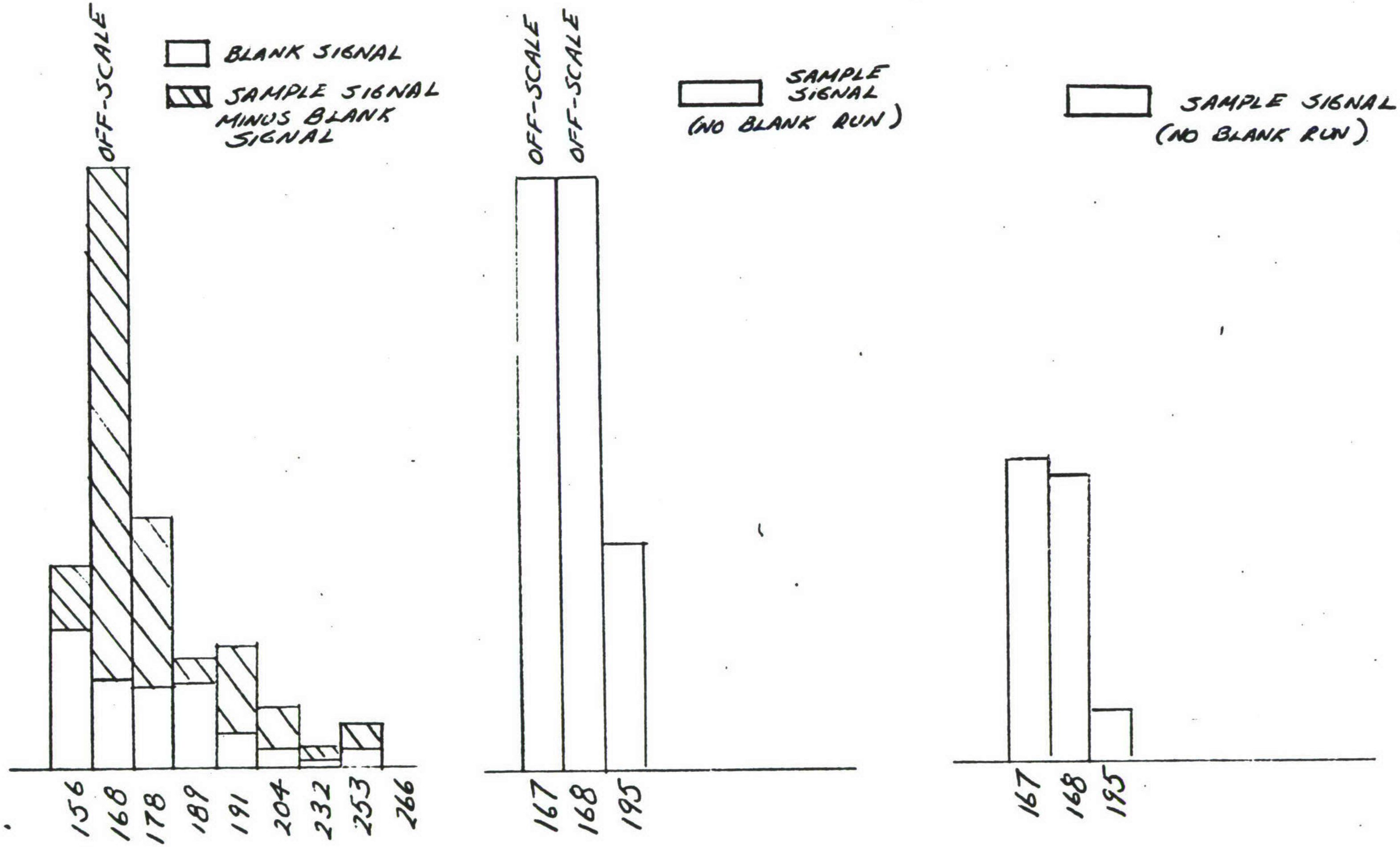


FIGURE. 27A 10 μl of 1:3 $CHCl_3$ EXTRACT OF pH 2 GASTRIC CONTENTS (SCH-2)

27B GASTRIC EXTRACT (SEC)

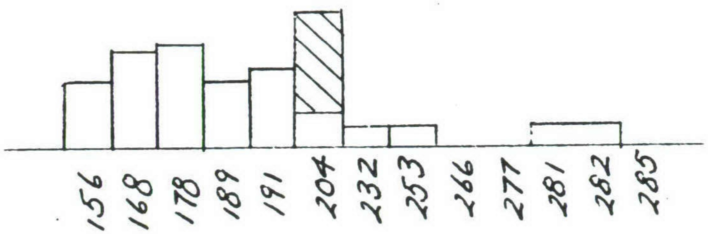
27C SECOBARBITAL STANDARD, pH 2 AQ. SOL'N

m/e	S/B	S-B
204	4.0	18

S/B = 1.0 FOR OTHER IONS

~~SAMPLE~~ BLANK SIGNAL

SAMPLE SIGNAL MINUS BLANK SIGNAL



28D'. 10μl CHLOROFORM EXTRACT OF SERUM (SCH-3)

m/e	S/B	S-B
204	7.7	40
282	1.3	1

S/B = 1.0 FOR OTHER IONS

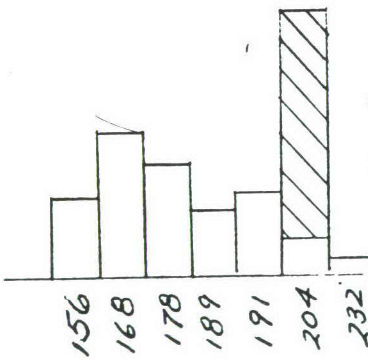
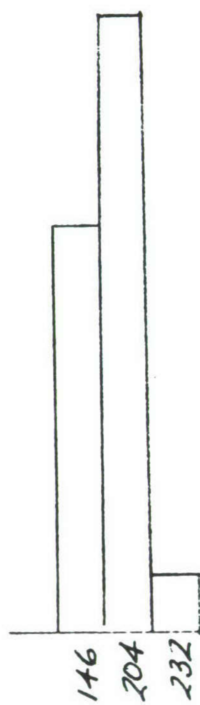


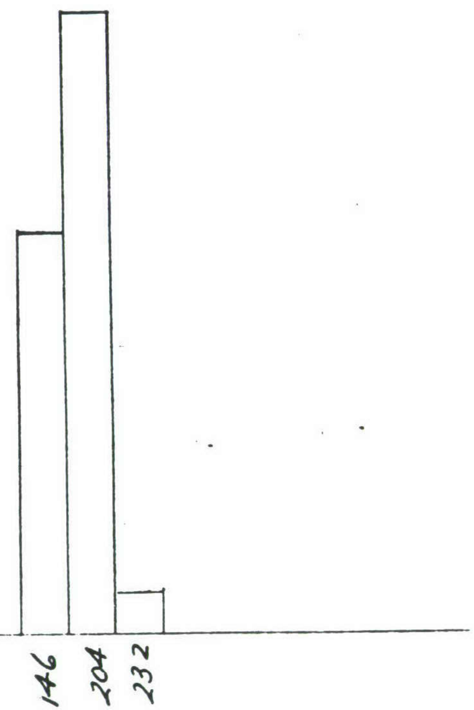
FIG. 28A 10μl URINE, ADJUSTED TO pH2 (SCH-3)

SAMPLE SIGNAL (NO BLANK RUN)



28B 10μl URINE (PHE)

SAMPLE SIGNAL (NO BLANK RUN)



28C 10μl 10^{-3}M pH2 AQ. SOL'N OF PHENOBARBITAL (2μg) (PHE)

m/e	S/B	S-B
276	1.4	4
277	1.5	4
278	1.2	2
283	2.1	15
284	6.7	46
285	9.0	64
286	4.4	17
294	1.3	2
298	1.3	3
299	1.5	3
300	1.5	3

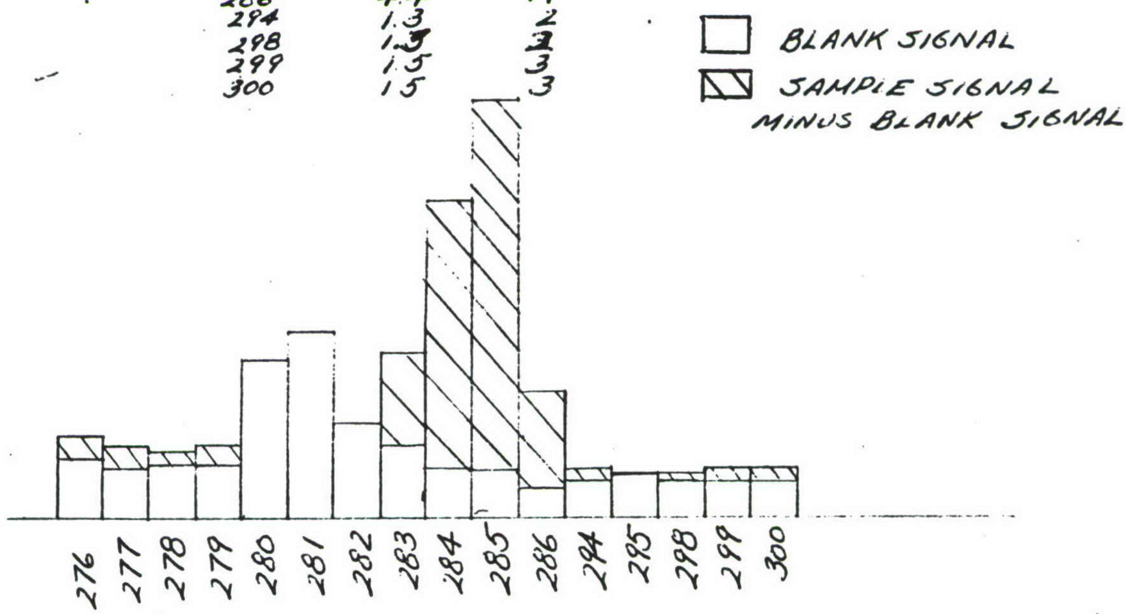


FIG. 29 10 µl OF 500 µl ACETONE SOLUTION OF EXTRACT OF 10 ml HYDROLYZED URINE
(NAR)

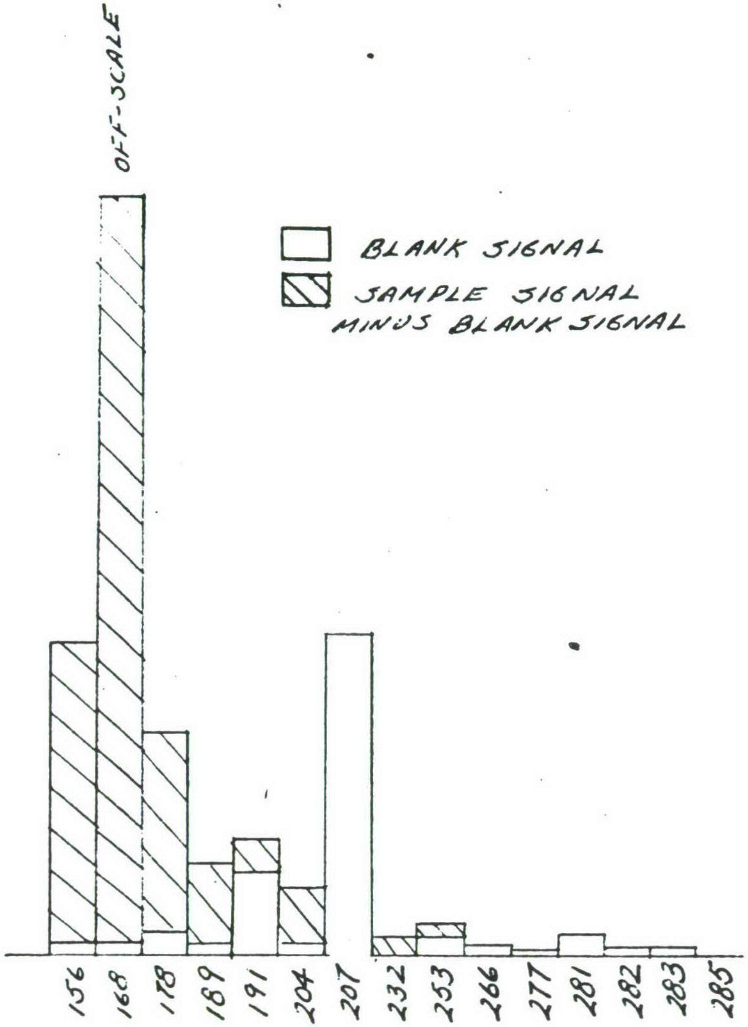
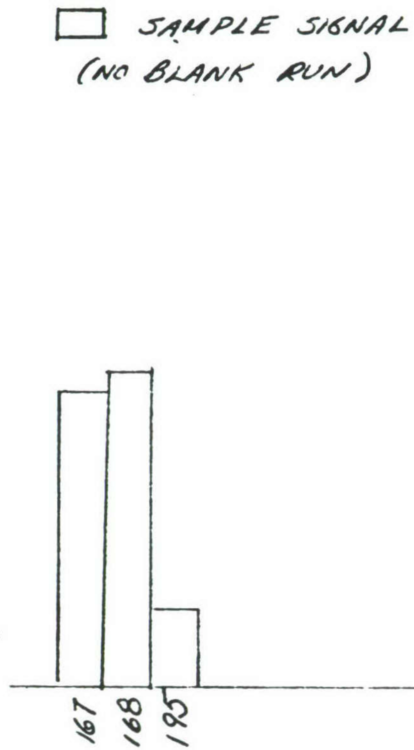
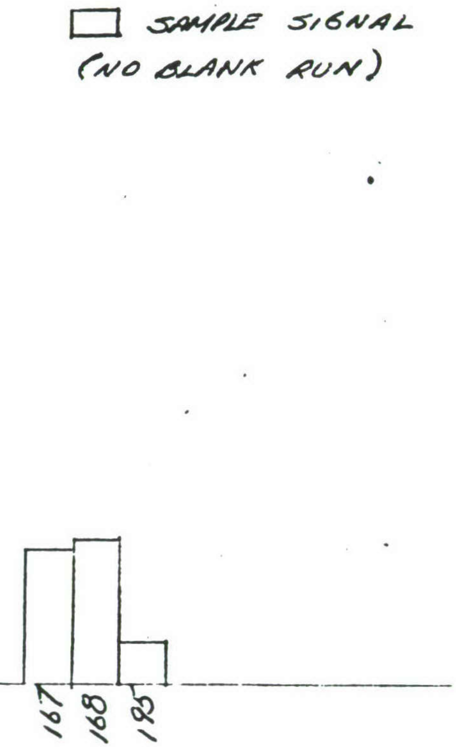


FIG. 30A 25μl pH2 ETHER EXTRACT OF URINE (1:1) (SCH)



30B URINE EXTRACT (SEC)



30C. AQ. pH2 SOL'N OF SECOBARBITAL (SEC)

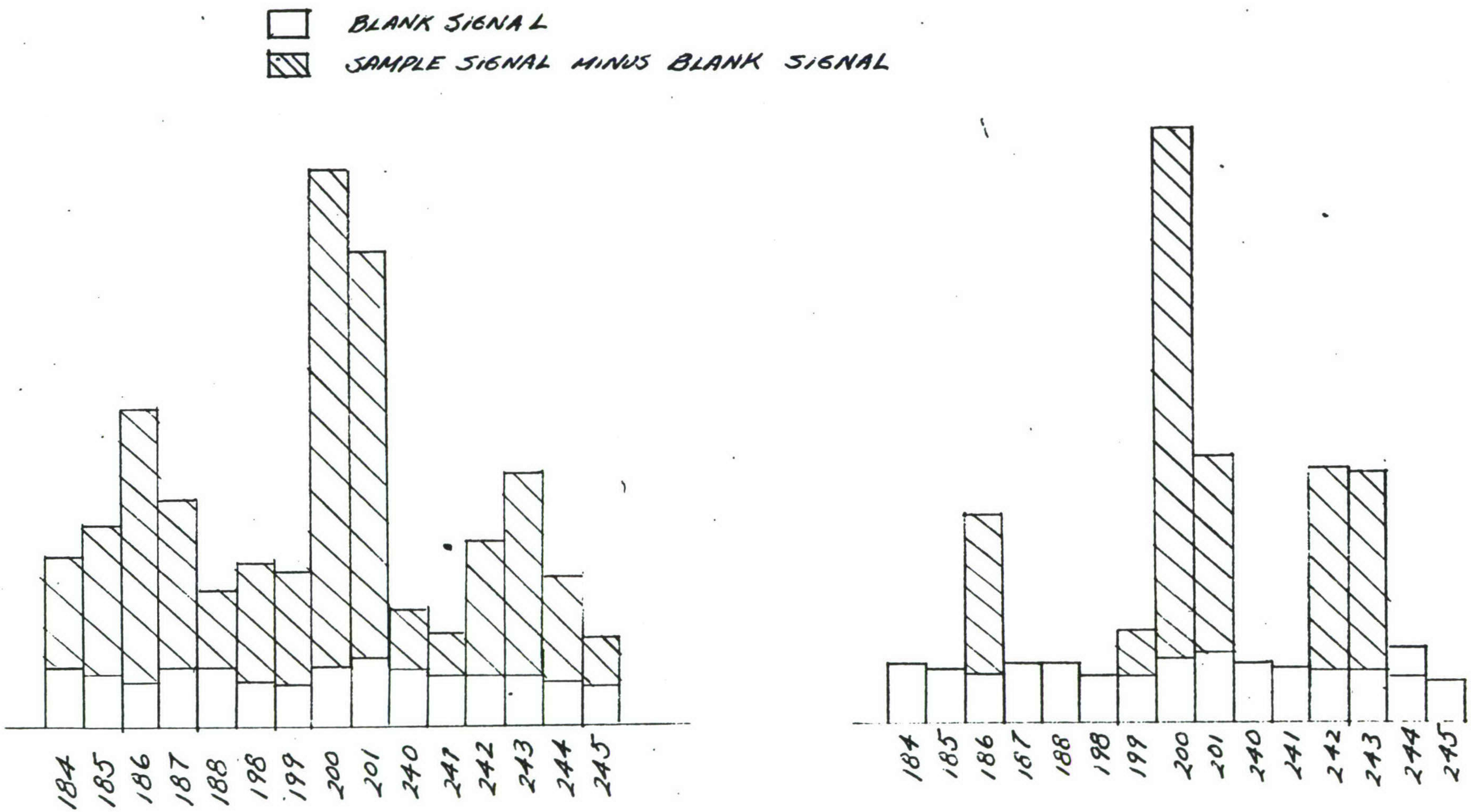


FIG. 31A 25 μl of 250 μl BASIC EXTRACT (ETHER 4:1) OF OVERDOSE URINE (PCP)

31B. STANDARD: SOL'N OF PHENCYCLIDINE IN ETHER (PCP)

VI. Rapid Drug Screening

The CVA-Mass Spectrometer system allows real time, multiple drug detection by monitoring for the high mass ions of the drugs of interest, using computer control of the quadrupole mass analyzer.

To demonstrate the speed capability of the CVA system, ten urine samples consisting of nine negative for drugs and one positive (urine spiked with phenobarbital to concentration of 0.5 mg %) were screened for acidic drugs using a SCH-type program.

For a sample population consisting of 10% drug positives, the demonstrated analysis time was 17 min/10 samples or $\sim$ 2 minutes per sample.

The analytical procedure, involving analysis of an ether extract of the urine is listed below:

Procedure:

Begin with ten screw cap vials of 4 ml urine each, 10 screw cap vials of 1 ml ether each.

(1) To each urine vial, add (syringe) 50 μl con. HCl to bring urine to pH2 (10 sec per sample).

(2) Add urine and ether to separatory funnel (10 ml), shake and allow phases to separate. Five minutes for 10 samples.

(3) Withdraw (syringe) 25 μl of upper (ether) phase and inject into CVA Mass Spectrometer system (5 sec per sample).

(4) Negatives. If drug peak (s) is not observed in 30 sec, inject next sample.

(5) Positives. If drug peak is observed (usually within 5-30 sec depending on particular drug's transit time through membrane system) on SCH program, switch system to particular drug subprogram (i.e. - PHE) and inject again. After 30 sec, flush with 25 μl acetone and wait 3 minutes for all drug to clear system. Time is thus 4 minutes per positive.

Timing	9 negatives	(a)	pH adjustment	100 sec
	1 positive	(b)	org-aq. partition	5 min
		(c)	syringe withdrawal system-injection and SCH - 9 negatives 1 positive	4 min 6 min
				~ 17 min
			(for 10 samples)	

The urine extract SCH profiles are shown in Fig. 32. The analyzer pressure was observed to increase from 7×10^{-7} to 9×10^{-7} torr in approximately 10 sec after injection and return to 7×10^{-7} torr in approximately 30-45 sec.

An alternative procedure, direct injection of urine after pH adjustment was run on the same sample population as above (Fig. 33). This procedure requires acetone flushing after every sample to minimize inlet, membrane and analyzer build-up. Although it is viable for drug overdose analysis it is not viable for high volume screening. Thus, the extract analysis is the preferred screening procedure.

In a fully developed CVA system one would (1) automate the extraction and injection steps, and (2) have the computer make the + or - decision based on memory-stored drug peaks.

The above steps involve modest development and will yield an automated, real time multiple drug analysis system with computer output.

VII. Detection of Heroin Abuse

Work at San Francisco General Hospital (SFGH) demonstrated detection of heroin abuse 3-4 days after an addict fix, utilizing CVA mass spectrometric analysis of morphine in urine. Suggestions for extension of CVA detectability beyond this period are made.

The significance of the CVA capability of screening for multiple drugs simultaneously is discussed for the specific situation of morphine-codeine analysis. The finding that urinary codeine level may be a "marker" of heroin addition is discussed.

Finally, CVA Mass Spectrometry is compared to other narcotic drug screen techniques.

A. Introduction.

The time period after heroin administration during which CVA Mass Spectrometry can detect morphine in an addict urine will depend on the following factors:

(1) addict dose.

(2) method of converting the highly polar morphine-glucuronide to a more volatile, membrane-soluble species amenable to CVA analysis.

  (a) acid hydrolysis to morphine

  (b) enzymatic hydrolysis to morphine

  (c) conversion to TMS ether or to a permethylated derivative

(3) sample background in m/e 285 region (molecular ion base peak or morphine mass spectrum).

An addict dose of heroin can be in the range 100 mg - 4 g/day. The major urinary metabolites of heroin are free and bound morphine (structures illustrated in Fig. 34). Renal excretion of free morphine ranges from 1 - 14% of dose and occurs in the initial eight hours after dose.[11] Over 50% of dose is excreted in the urine as bound morphine, and of this total bound morphine, 50% is excreted within eight hours and 90% within 24 hours. Traces of bound morphine have been detected up to 48 hours.

B. Studies at San Francisco General Hospital.

Studies by Udo Boerner[12a] (SFGH) indicated bound morphine levels of 0.25 - 0.50 μg/ml in the urine of heroin addicts 3 - 4 days after a fix. Analysis was run by thin layer chromatography of acid-hydrolyzed urine.

Fifteen Heroin Detoxification Unit admission urines and ten Methadone Maintenance patient urines were analyzed by CVA Mass Spectrometry at SFGH.[12b] The ten admission urines gave strong positive morphines as seen by the intense molecular ion at m/e 285 (Fig. 35A). Of the ten methadone

[11] P. Paerregard, Acta Pharmacol (Kobenhaven), 14, 53.

[12a] Private communication.

[12b] Admission urines are obtained from addicts applying for the detoxification program. If accepted to the detoxification unit (on the basis of a ⊕ urinary morphine) the patient is then put on a regimen of methadone and must submit a urine sample daily for drug analysis. Such samples analyzed here were taken three days after patient admission.

clinic urines, eight were negative and two had trace positives (Fig. 35B) indicating a residual trace of morphine from a pre-clinic fix (3-4 days earlier). These results were confirmed by Toxicology Lab Analysis (TLC) by Mr. Boerner.

It is notable that whereas Mr. Boerner ran TLC on the chloroform extract of 20 ml urine, CVA was run on 1/10 of the extract. This reflects the fact that while the TLC detection limit is ~ 5 μg, that of CVA is ~ 0.5 - 1.0 μg.

C. Suggestions for Future Analytical Work.

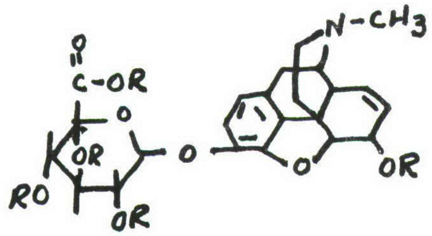
It is significant that the CVA detection limit for morphine is due to signal to background rather than signal to noise ratio. The bulk of this background is due to products of acid hydrolysis of normal urinary components. Thus, use of an alternative method of conversion of bound morphine yielding a reduced background relative to acid hydrolysis should increase sensitivity and thus increase the time period during which heroin abuse is detectable.

Enzymatic hydrolysis with β-glucuronidase is a simpler and cleaner method than acid hydrolysis. Although enzymatic hydrolysis requires incubating (37°C) the sample overnight, the procedure is quite simple: Buffer the urine to pH 5.2, add β-glucuronidase, incubate. This procedure should lend itself to high volume screening if one can wait to have sample results the day after urine collection.

After incubation, adjust the urine to pH 9, extract into chloroform and inject into the CVA Mass Spectrometer. Analysis time is two minutes (extraction) plus 30 seconds (morphine negative) or four minutes (morphine positive).

An alternative would be conversion of bound morphine to a relatively non-polar (therefore more volatile, more membrane-soluble) derivative. Trimethylsilyl ether[13] or permethylated ether derivatives appear likely.

[13] G. Martin and J. Swinehart, Anal. Chem., 38, 1789 (1966).



$$R = -\overset{CH_3}{\underset{CH_3}{Si}}-CH_3 \text{ or } CH_3$$

Silylation of a chloroform extract of urine with BSA [bis (trimethylsilyl) acetamide] would take about five minutes, followed by injection into the CVA Mass Spectrometer and monitoring of suitable high mass fragments of the morphine glucuronide TMS ether derivative.

The enzymatic hydrolysis and non-polar derivatization approaches appear promising for screening of urines collected several days after heroin abuse and would be a critical part of future CVA Mass Spectrometry work in rapid drug screen applications.

D. Significance of CVA Capability of Screening Multiple Drugs Simultaneously.

In detecting trace morphine in a urine screen, one cannot be satisfied that this result in itself indicates heroin abuse. Codeine, a commonly used therapeutic analgesic (in Empirin #3) undergoes metabolic O-demethylation to morphine. The ratio of urinary codeine to morphine (both present predominantly in bound form) is approximately 7:1,[14] with morphine representing 5-10% of dose.

Thus, one should simultaneously monitor morphine <u>and</u> codeine to avoid mistaking codeine use (or abuse) for heroin abuse.

Evidence has been collected at SFGH that codeine is a "marker" of heroin addiction. Fifty-six of sixty-four morphine positive Heroin Detoxification Unit admission urines were found to contain urinary codeine levels of 5-10% relative to morphine levels. Twenty of the samples were analyzed by CVA Mass Spectrometry (Fig. 35A) and TLC with results correlating. The remainder were analyzed by TLC (Mr. Boerner).

[14] T. K. Adler, et al, J. Pharmacol, <u>114</u>, 251 (1955).

Eighteen samples of street heroin (obtained from Bay Area crime labs) were analyzed by TLC and CVA Mass Spectrometry. One sample contained approximately 4% codeine and traces of mono-acetyl codeine. The other 17 samples did not contain detectable codeine.

It seems reasonable to assume that most heroin available and used by the heroin addicts studied is codeine-free. Thus, the significant codeine levels present in the addict urines reflect biotransformation of morphine to codeine.

One non-addict subject ingested 100 mg morphine sulfate (analyzed by TLC, CVA to be codeine-free) and provided a pooled 24-hour urine. Codeine was found by TLC and CVA at trace level (<1.0% relative to morphine).

The increase in codeine formation in heroin addicts indicates alteration of addict metabolism by chronic heroin abuse. Two possible metabolic schemes are suggested to account for this abnormal metabolism. If the major biotransformation pathways are:

1. heroin $\xrightarrow{k_a}$ 6 monoacetyl morphine (MAM)
2. MAM $\xrightarrow{k_b}$ morphine
3. morphine $\xrightarrow{k_c}$ morphine glucuronide (glucurony transferase)
4. morphine $\xrightarrow{k_d}$ codeine (O-methylase)
5. codeine $\xrightarrow{k_e}$ codeine glucuronide
6. codeine $\xrightarrow{k_f}$ morphine (O-demethylase)

Then schemes I, II are postulated:

Scheme	Non-addict	Addict
1	$k_c >> k_d$	k_c reduced, k_d constant or increased, $\therefore k_c > k_d$
II	$k_f > k_d$	k_f reduced and/or k_d increased, $\therefore k_d > k_f$

An added factor in this observation is the possibility of liver dysfunction in the heroin addict. Sixty percent of the addicts had contracted hepatitis during the abuse period.

Thus, simultaneous monitoring of morphine and codeine as performed by CVA Mass Spectrometry (monitor morphine molecular ion at m/e 285 and codeine molecular ion at m/e 299) may be able to distinguish the experimenter or new user from the hard core addict.

E. Comparison to Alternate Drug Screen Techniques.

Simultaneous differentiation of morphine and codeine is possible using:

(1) CVA Mass Spectrometry.

(2) Gas chromatography - mass spectrometry (GC-MS).

(3) Gas chromatography (GC).

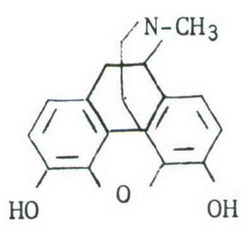
(4) Thin-layer chromatography (TLC).

Whereas CVA analysis time for a hydrolyzed urine extract is 30 seconds, that of GC and GC-MS is 30 minutes. The latter time periods are not amenable to high volume screening. TLC analysis time is 30-45 minutes but multiple samples can be run on the same chromatoplate.

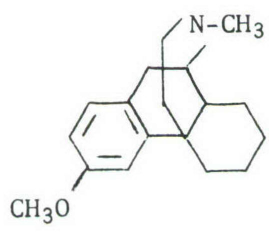
TLC is subject to false positives at trace drug levels. The TLC detection limit of ~ 5 μg morphine places a limit on abuse detection. CVA detection limit is ~ 0.5 μg morphine.

Non-differentiating techniques such as FRAT and UV spectrophotometry are subject to false positives from other narcotics. Examples are dextromethorphan, meperidine, diphenoxylate, and codeine.

Dextromethorphan (Romilar), a commonly used anti-tussive is a structural analogue of morphine in that it contains a phenanthrene-type nucleus and a piperidine ring with a basic tertiary nitrogen atom.



Morphine



Dextromethorphan

A normal adult dose of dextromethorphan is 20 mg every eight hours. Dextromethorphan is excreted in the urine predominantly as the glucuronides of unchanged drug and its N- and O-demethylated metabolites.[15]

Little is known of dextromethorphan excretion rate. Assuming an excretion rate similar to that of the other opiates (i.e. morphine) - 70% of dose/24 hours, an average urinary level of dextromethorphan and metabolites is then

$$\frac{0.70 \times 20 \times 10^3\ \mu g \times 3 \text{ per day}}{1500 \text{ ml urine per day}} = 28\ \mu g/ml$$

Urinary bound morphine levels three days after a fix are 0.25 - 0.5 μg/ml. In a test such as FRAT, sensitivity to dextromethorphan and its metabolites must be $< \frac{1}{100}$ than that to bound morphine to avoid interferences. Relative FRAT sensitivity of dextromethorphan vs. morphine is reportedly 1/200. Thus interference problems are expected only beyond three days after fix.

Dextromethorphan with a reportedly intense molecular ion at m/e 271 (31% of base peak intensity) is readily differentiated from morphine by CVA Mass Spectrometry.

Meperidine (Demerol), a commonly prescribed narcotic analgesic, is excreted in the urine to the extent of $\sim$ 55% of dose/24 hours as unchanged drug and several metabolites.[16]

Assuming this excretion as averaged over 24 hours, for a normal dose of 100 mg, an average urinary level of meperidine and its metabolites is then

$$\frac{(0.55)(100 \times 10^3\ \mu g)}{1500} = 37\ \mu g/ml$$

In screening for a morphine level of 0.25 μg/ml, a technique such as FRAT must be $< \frac{1}{150}$ as sensitive to meperidine and its metabolites than to

[15] E. L. Way and T. K. Adler, Biological Disposition of Morphine and Its Serogates, World Health Organization, P. 59, (1962).

[16] N. Plothekoff et al, J. Pharmacol, 117, 414 (1956).

morphine. Relative FRAT sensitivity of meperidine vs. morphine is 1/26. Thus interferences are expected.

Meperidine, with an intense molecular ion at m/e 247 (38% of base peak intensity), is readily differentiated from morphine by CVA Mass Spectrometry.

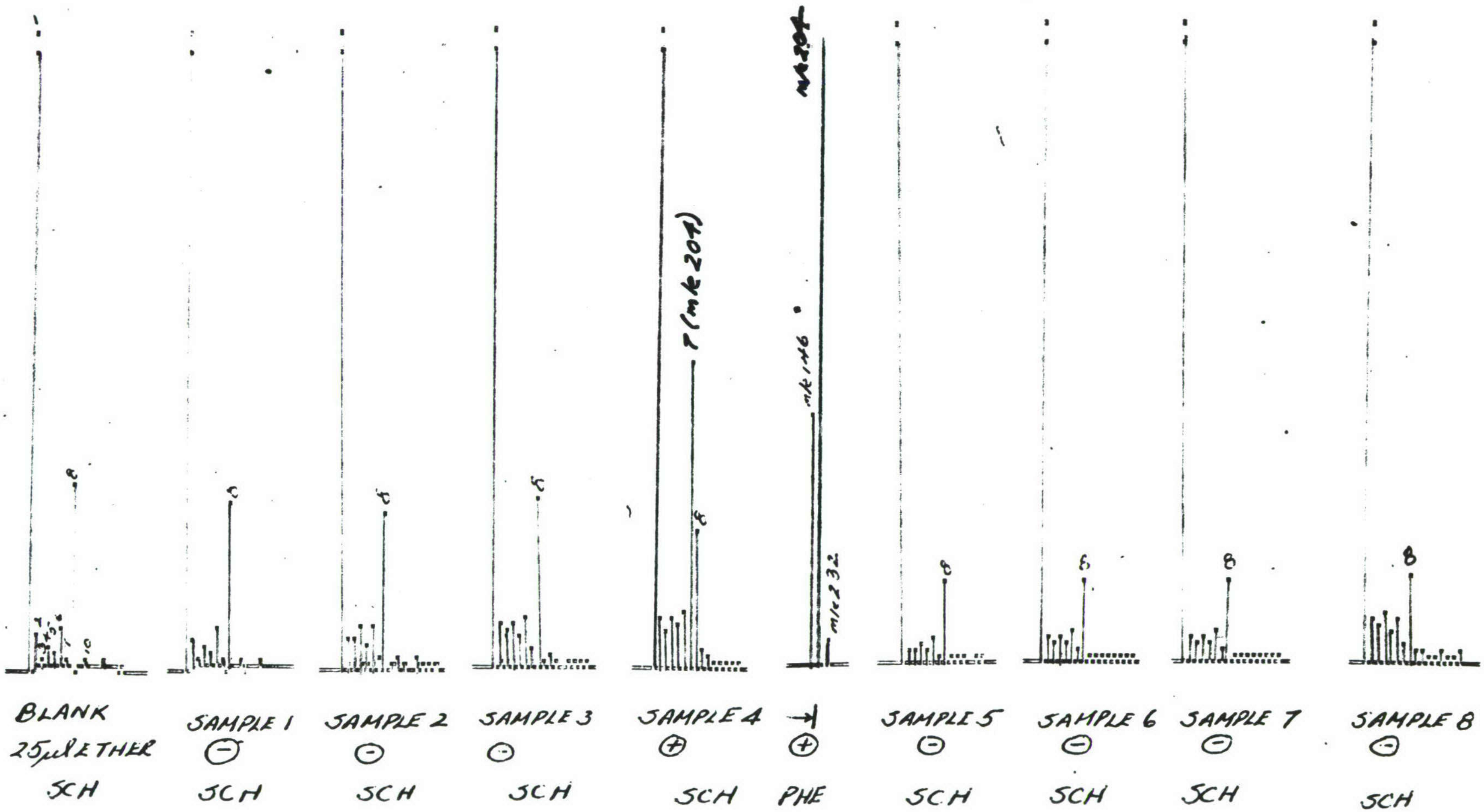
FRAT is five times more sensitive toward codeine than morphine. Thus codeine use will result in interferences in morphine screening.

F. Tentative Detection of Heroin Abuse by Analysis for Drug Adulterants. Heroin is frequently cut with quinine, procaine and phenobarbital. The SFGH Toxicology Lab found phenobarbital in 20 of 74 heroin admission urines.

In our previous work on heroin addict urines we had used basic (pH 9) extracts of urine and thus did not detect the acidic phenobarbital. However previous work with CVA Mass Spectrometry did demonstrate sub-microgram sensitivity to phenobarbital with positive detection on therapeutic and overdose urines. Phenobarbital level in cut street heroin is approximately equal to that of the heroin. Thus, the phenobarbital dose would be $\sim$ 100 mg-4g/day.

Phenobarbital, a long acting barbiturate, is excreted unchanged slowly ($\sim$ 50% of dose) in the urine over a period of several days. Thus, monitoring for phenobarbital as a tentative indication of heroin abuse (phenobarbital alone is also a common drug of abuse) may allow one to detect the heroin abuser beyond the time period in which urinary morphine levels have become undetectable.

Since CVA Mass Spectrometry is capable of multiple drug screening, analysis of drugs used to cut heroin is feasible.



PEAK	m/e	
1	58	
2	156	Pentobarbital
3	168	Secobarbital
4	178	
5	189	Glutethimide
6	191	
7	204	Phenobarbital
8	207	
9	232	Chlorinated Phenothiazine
10	253	Oxazepam
11	266	Fluorinated Phenothiazine
12	277	Methadone Metabolite
13	281	

PEAK	m/e	
14	282	Chlordiazepoxide
15	283	Diazepam
16	285	Morphine

FIG. 32 URINE EXTRACT DRUG SCREEN

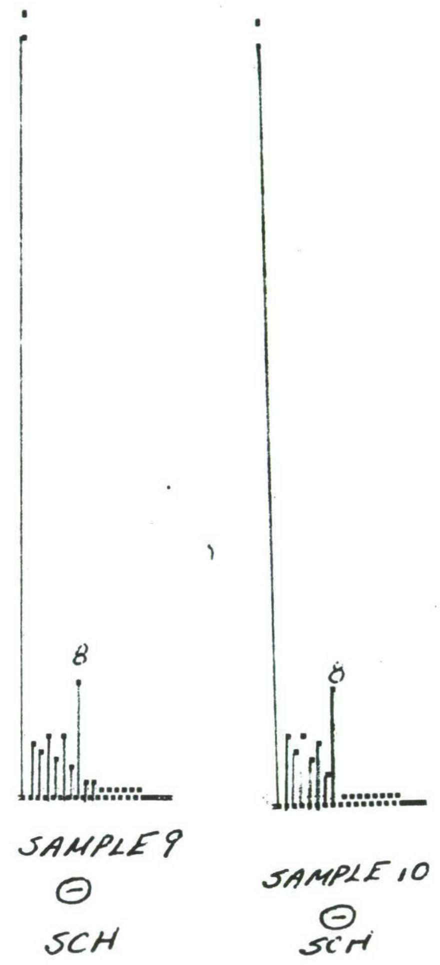
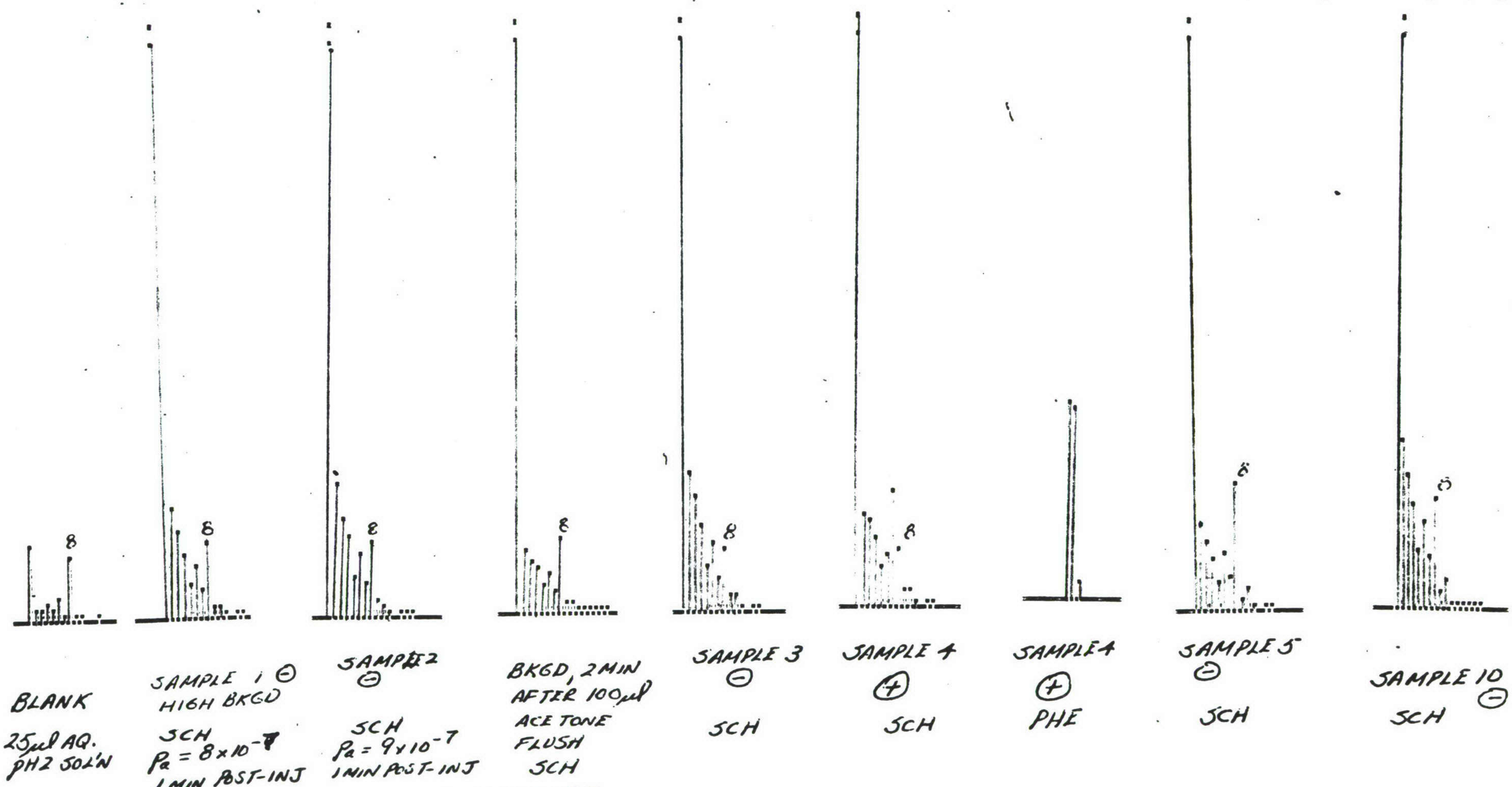


FIG. 32 URINE EXTRACT DRUG SCREEN (CONTINUED)



P_a = ANALYZER PRESSURE
ACETONE FLUSH AFTER SAMPLES 2, 4, 6, 8.

FIG. 33 DIRECT INJECTION OF URINE

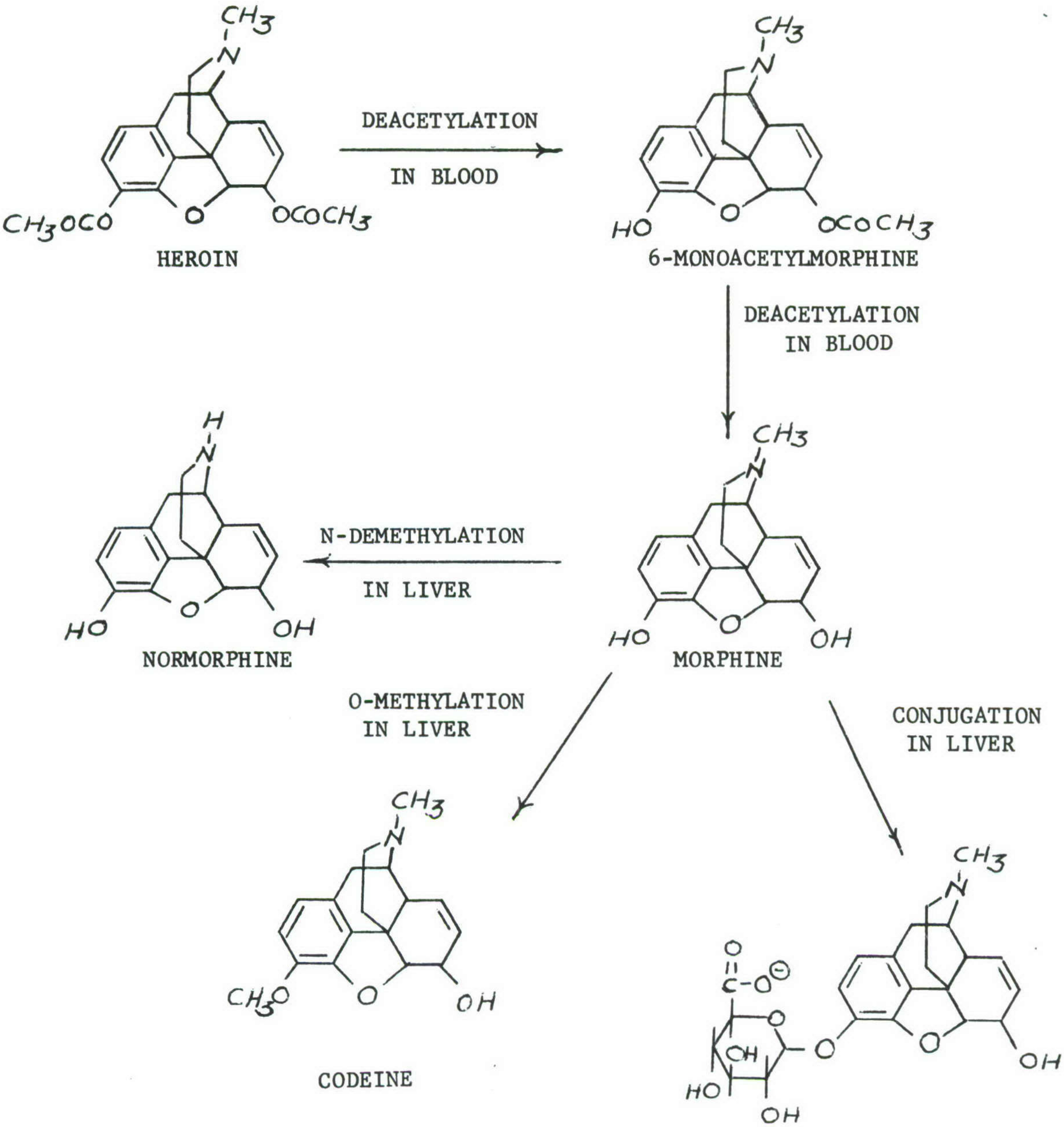


FIG. 34 METABOLISM OF HEROIN

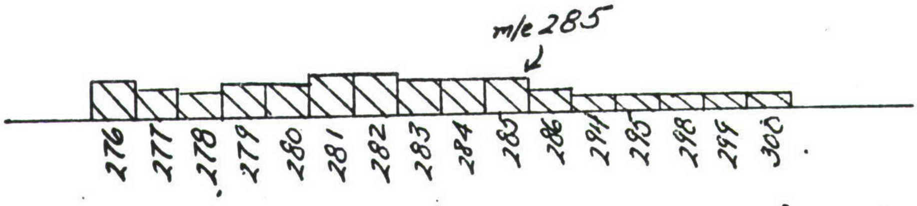


FIGURE 35C 10µl OF 100µl EXTRACT OF 20 ml HYDROLYZED URINE OF METHADONE MAINTENANCE PATIENT → MORPHINE NEGATIVE
URINE COLLECTED 3-4 DAYS AFTER LAST FIX

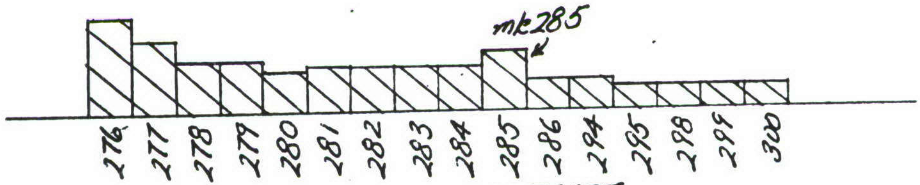


FIGURE 35B 10µl OF 100µl EXTRACT OF 20 ml HYDROLYZED URINE OF METHADONE MAINTENANCE PATIENT
→ TRACE MORPHINE POSITIVE
URINE COLLECTED 3-4 DAYS POST-FIX

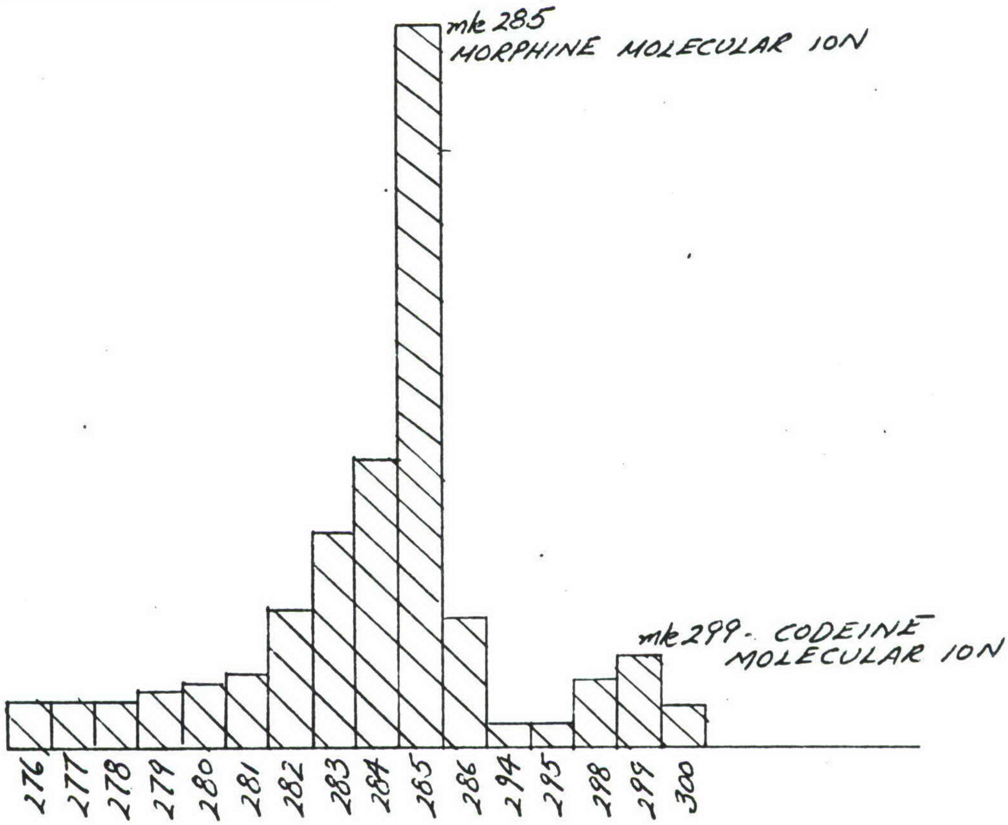


FIGURE 35A 10µl OF 100µl EXTRACT OF 20 ml HYDROLYZED URINE OF HEROIN ADMISSION PATIENT

VIII. Detection of Drugs in Saliva, Breath, Skin Wipings

The results described above in this report were obtained from urine, blood or gastric samples. As part of this work samples obtained from saliva, breath and skin wipes were tested and analyzed. These biological media are easy to collect and are of special interest to LWL.

Work done at Varian consisted of a comparison of a urine and saliva sample collected from a male patient undergoing phenobarbital withdrawal at SFGH (see Section IV-1). The patient dose of 700 mg/day represented that of a tolerant barbituate addict. Analysis of 10 μl urine, monitoring m/e 204, indicated a phenobarbital level of ~ 20 mg % (Fig. 36 B). Analysis of one ml saliva (work-up Procedure 1 in Appendix) indicated no detectable phenobarbital (Fig. 36A).

The phenobarbital detection limit is $\lesssim$ 100 ng and, assuming ~ 50% work-up efficiency, the salivary drug level is < 200 ng/ml. The relative urinary/salivary levels are thus 200 μg/ml versus < 0.2 μg/ml or a ratio of $>10^3$ to 1.

At SFGH, we were able to obtain a urine (collected by catheter) and acetone skin wipe (see Procedure 2 in Appendix) of a poisoning case. Analysis of 10 μl urine monitoring m/e 232 indicated ~ 50 mg % chlorinated phenothiazine (Fig. 37). Analysis of the acetone skin wipe indicated no detectable phenothiazine. Chlorinated phenothiazine detection limit is $\lesssim$ 1 μg and thus the relative urinary/skin drug levels were 500 μg/ml versus < 1 μg/10 in.^{2} forearm.

Urine, breath (Procedure 3 of Appendix), saliva and skin wipe samples were collected from fifteen Heroin Detoxification Unit admission patients. Urines were acid-hydrolyzed (to split the morphine glucuronide) and extracted. Positives at a level of ~ 10-50 μg/ml (1-5 mg %) were obtained by CVA Mass Spectrometry urine analysis, monitoring the morphine molecular ion at m/e 285 (Fig. 38).

The breath, saliva and skin wipe samples did not contain detectable morphine. This would indicate levels definitely below 1 μg/15 minutes breath, 1 μg/ml saliva, 1 μg/10 in.^{2} skin respectively.

A comparison of a breath and a urine scan is shown in Fig. 38.

The results on saliva agree with those of Oberst,[17] who in a study of six heroin addicts on a daily dosage between 105-4200 mg morphine, failed to detect morphine in saliva. Oberst's analytical technique was sensitive to 30 μg and his sample volumes were 20-260 ml. One can then calculate that the saliva levels were definitely < 1 μg/ml in some cases, < 0.1 μg/ml in others.

A critical factor in such studies may be time after dose at which the sample is collected. This is not known in the SFGH work. For example, it is possible that < 1 μg/ml salivary drug levels exist within 1-2 hours of dose but that the level is considerably reduced at later times. Such an effect was noted in studies reported on detection of meperidine in saliva (intramuscular administration) at 3.5 - 6 μg/ml levels,[5] <u>1-2</u> hours after 100 mg dose.

Oberst[17] did note traces of free morphine in the perspiration of heroin addicts. Levels were 2-5 μg/ml for three subjects. The volumes collected (58-215 ml) suggest an artifical stimulation of the subject. Perhaps the acetone skin wipe collection would be more successful if done on a perspiring subject.

Oberst's success in detection of morphine in perspiration gives some hope for success in skin wipe analysis. It is possible that such success (assuming the morphine to be present on the skin) will require a more efficient collection procedure than the acetone skin wipe.

Dr. James Arnold of Varian has suggested use of a dimethylsilicone membrane to wipe the skin, followed by insertion of the membrane into the heated inlet.

The breath results are not surprising in that morphine is an extremely non-volatile molecule (mp 230°C) due to its large molecular size and high polarity.

[17] F. W. Oberst, J.Pharmacol, <u>74</u>, 37 (1942).

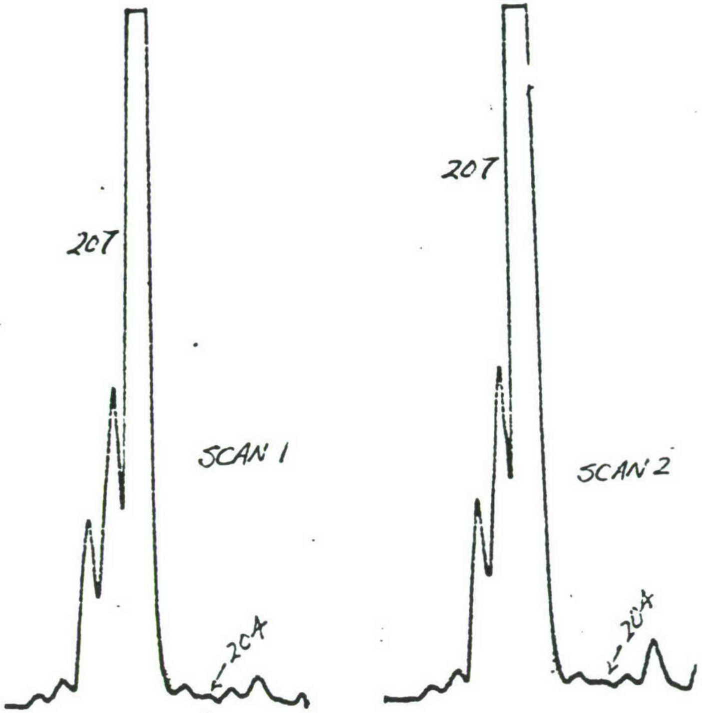


FIGURE 36A
10 SEC SCANS
25 µl EXTRACT OF
1 ml SALIVA
PHENOBARBITAL
WITHDRAWAL PATIENT

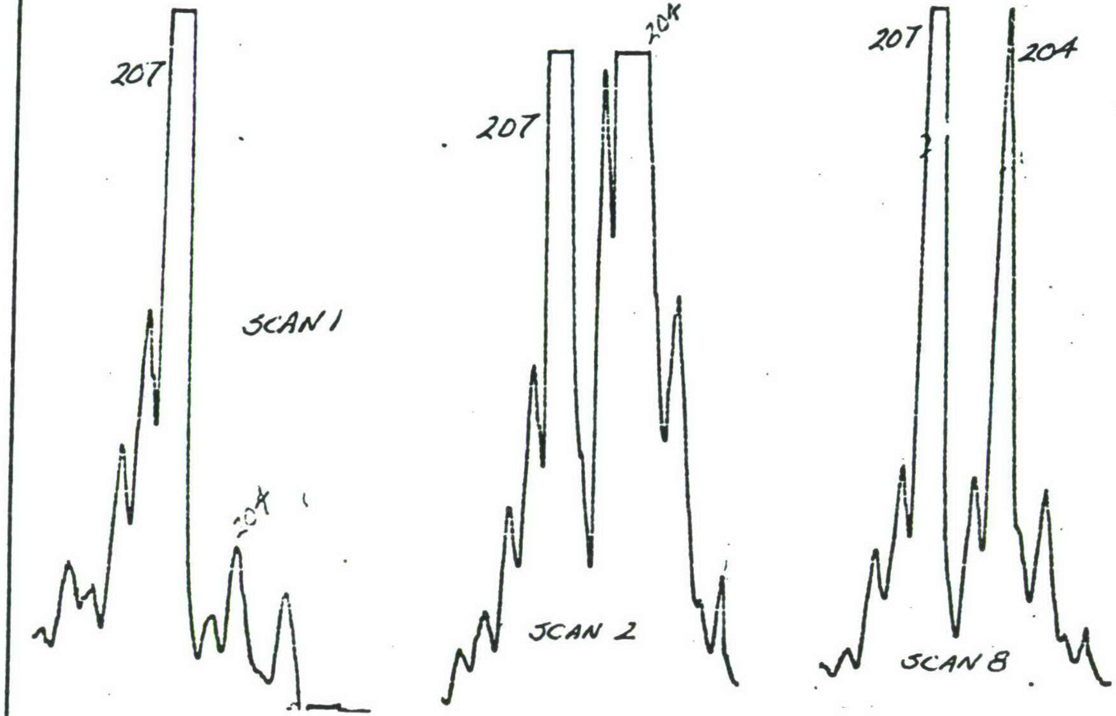


FIGURE 36B
25 µl URINE, ADJUSTED TO
pH 2
-DIRECT INJECTION

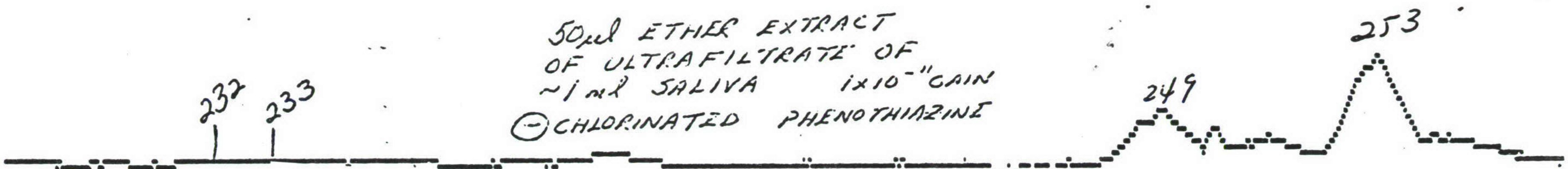


FIG. 37C

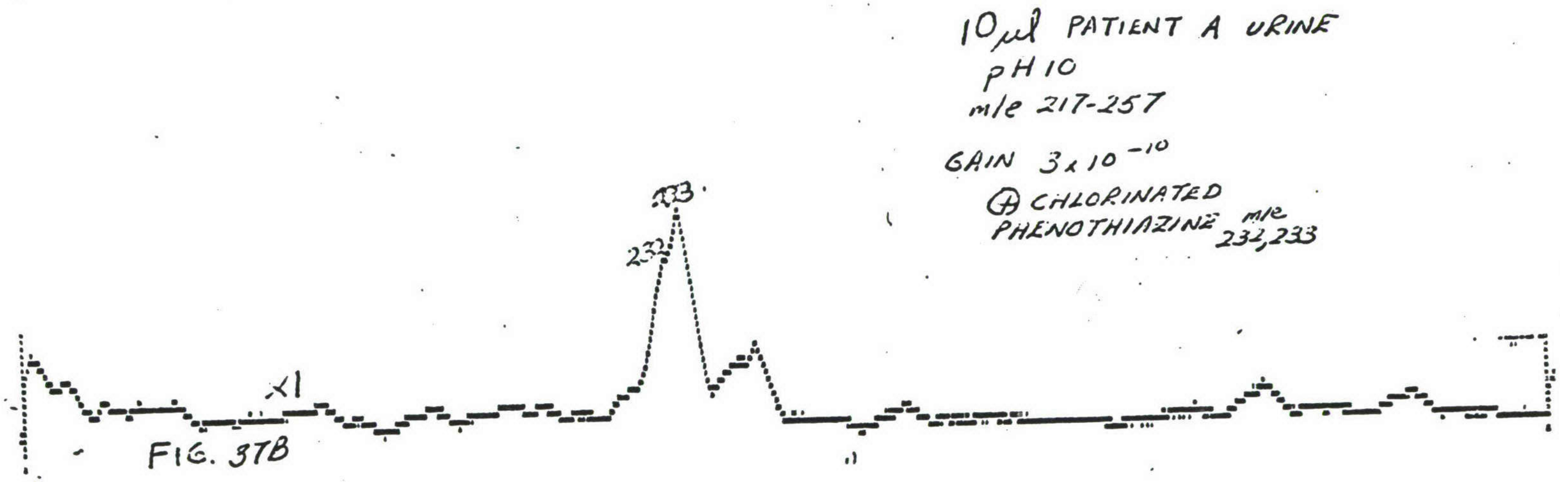


FIG. 37B

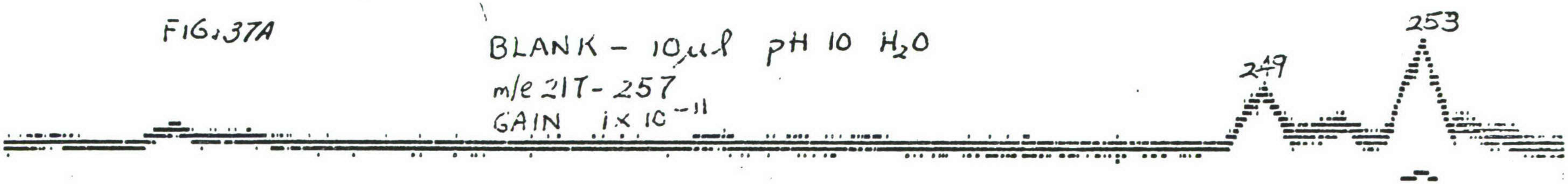


FIG. 37A

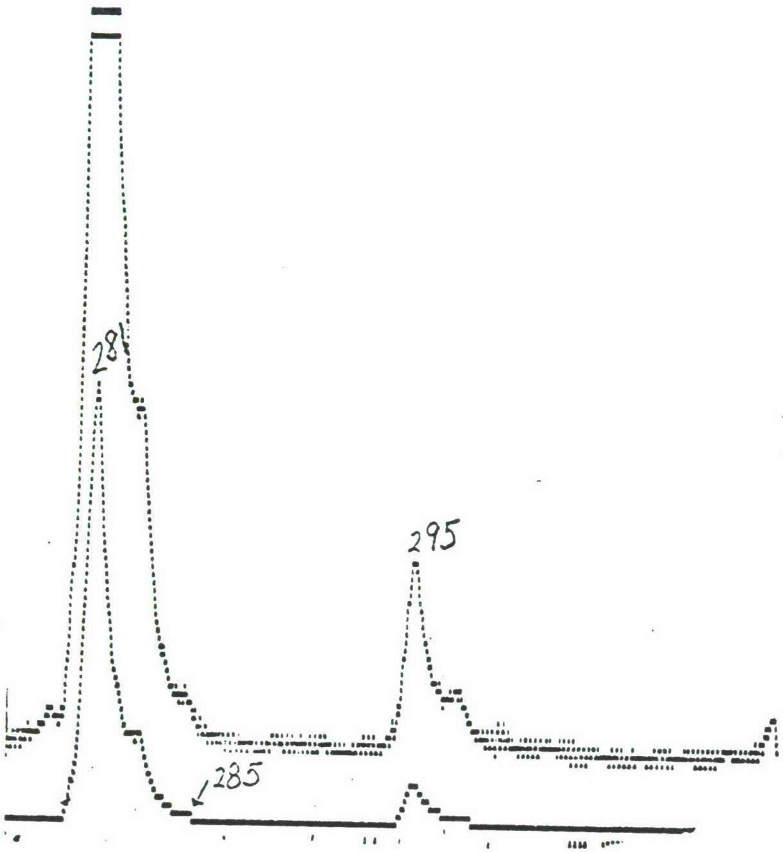


FIG. 38A
HEROIN ADMISSION
BREATH SAMPLE
30μl WORKUP
mk 281, 295 ⇒ SILICONE

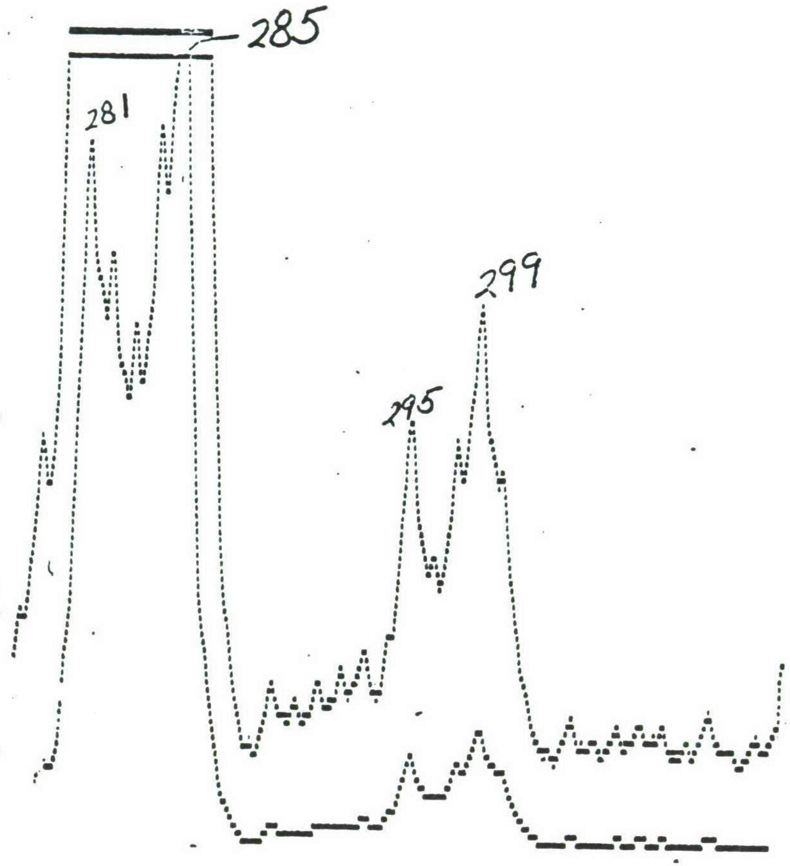


FIG. 38B
10μl HYDROLYZED
URINE EXTRACT

Its polarity and thus low lipid solubility would inhibit its transfer through body membranes (i.e., lung or skin).

IX. Detection of Methadone Use - Time Period After Use

LWL (Mr. Clay McDowell) requested a study of the time period over which CVA Mass Spectrometry could detect urinary morphine as an indicator of heroin or morphine abuse. It was not possible to obtain hospital permission to run such a controlled (dose, time) experiment on a human subject. However, the San Francisco General Hospital Methadone Maintenance Program (Dr. Arthur Weinberg, Director) was willing to provide a methadone maintenance patient to ingest a 60 mg methadone dose and provide urines up to 26 hours. At this point the patient took another dose (on a daily regimen) and thus the experiment terminated.

Methadone is a long-acting narcotic with abuse potential. The drug binds strongly to tissue protein and reportedly exhibits a slow kinetic decay in the plasma.

Analysis of urine extracts[18] indicated:

(1) Unchanged drug is detectable, using the M-15 ion at m/e 294, up to 13-1/2 hours. Maximum level occurs at four hours.

(2) Urinary excretion of the demethylated metabolites of methadone, monitored at m/e 276, 275, 264, is relatively slow and total metabolite level fairly constant over the 26-hour period.

(3) Urinary metabolite levels at 26 hours are $\geq$ 20 times the detectability of the system.

These observations would indicate ready detection of methadone use for at least several days.

An additional observation is that the intense peaks observed at m/e 275, 260 may represent a metabolite as yet unreported in the literature.

[18] 10 ml urine adjusted to pH2, washed with 10 ml ether, then extracted with 10 ml chloroform. The methadone salt is chloroform-soluble. The chloroform phase was evaporated to 1 ml under a heat gun and made basic with one drop ion NH_4OH prior to 25 μl injection.

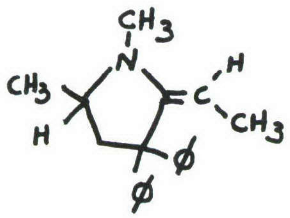
The CVA scans at 1/2 hour and 26 hours are shown in Figures 39 and 40. The mass region m/e 160 to 300 was scanned every three seconds during the two-minute period representing transmission of the drug and metabolites through the membrane. A scan at ~ 25 seconds represents approximate intensity maxima of the drug and metabolite peaks.

Although the diphenylmethane fragment ions at m/e 165, 178-180 are the most intense drug and metabolite ions, the ions in the m/e 260-294 range are less subject to possible interference either from normal urinary components or other drugs and are thus chosen to monitor methadone use.

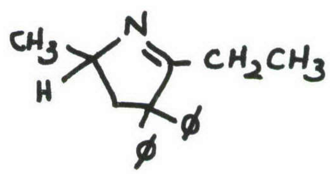
The key peak assignments are:

I.	m/e	294	M-15 (CH_3) ion of methadone
II.	m/e	277	M+ of cyclic N-demethylated methadone metabolite
		276	M-1
		262	M-15 (CH_3)
III.	m/e	275	M+ of cyclic N-demethylated, -2, 3-dehydromethadone metabolite
IV.	m/e	264	$(M+1)^+$ of N, N-didemethylated methadone metabolite

The metabolites II and IV have been reported[19,20] in the literature. Both metabolites had no pharmacological activity.[3]



II. MW 277



IV. MW 263

II should give a characteristic pyrrolidine spectrum: an intense molecular ion and M-R_1, M-R_2 ions where R_1, R_2 are the 2-substituents of the pyrrolidine ring.[21] These ions are m/e 277, 276, 262.

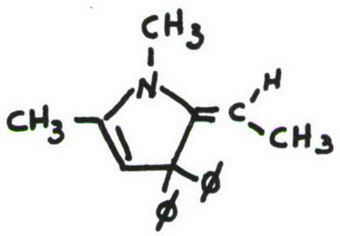
[19] A. Beckett et al, J. Pharm Pharmac. 20, 754 (1968)

[20] A. Pohland et al, J. Med. Chem. 14, 194 (1971)

[21] Q. Porter and J. Baldas, Mass Spectrometry of Heterocyclic Compounds, Wiley-Interscience, New York, 1971.

IV will not give $M-R_1$, $M-R_2$ ions due to the endocyclic double bond at positions 1, 5. The intense m/e 264 ion is tentatively assigned to the $(M+H)^+$ or M+1 quaternary ammonium ion of IV.

The peaks at m/e 275, 260 cannot be explained as fragments of I, II or IV. These peaks are tentatively assigned to structure III.



MW 275

This metabolite has not yet been reported in the literature. It can either be:

(1) a true metabolite

(2) a thermal oxidation product of metabolite II (reaction at CVA 250°C inlet or in the ion source).

One observes an increase in the m/e 275, 260 ions relative to the m/e 277, 264 ions at long post-dose time periods. This cannot be explained by (2).

A literature study[20] of urinary excretion of methadone and its metabolites in man indicated a ratio of about 2:1 of metabolite II to methadone I in a 24-hour pooled urine and only a trace of the didemethylated metabolite IV. The study was done on <u>one</u> subject, <u>one</u> dose.

Our study of one subject indicates significant amounts of metabolite other than IV. Further studies are indicated.

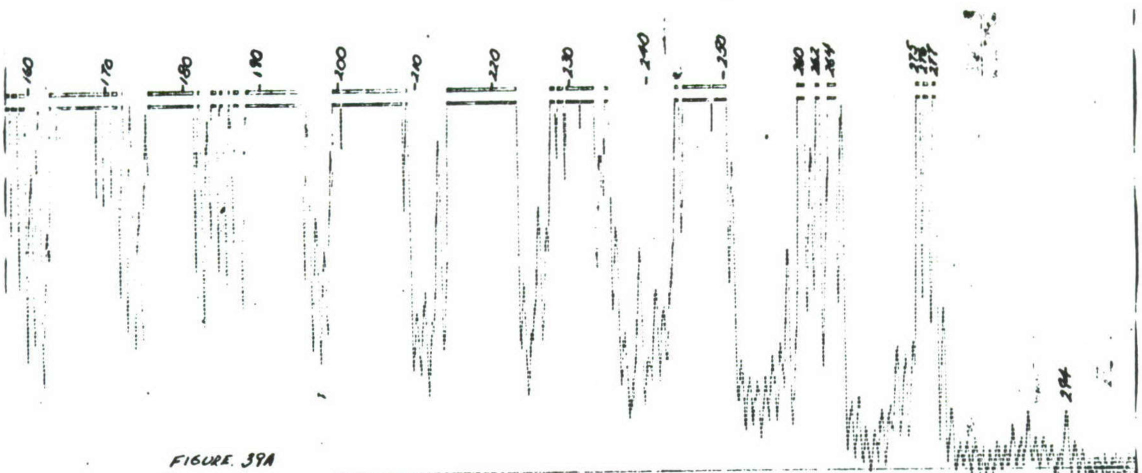


FIGURE 39A
1/2-HR. URINARY EXTRACT
25 µl OF 1 ML. CHLOROFORM EXTRACT OF 10 ML. URINE
1×10^{-10} GAIN
10 sec post-injection
T_i 250 T_m 180

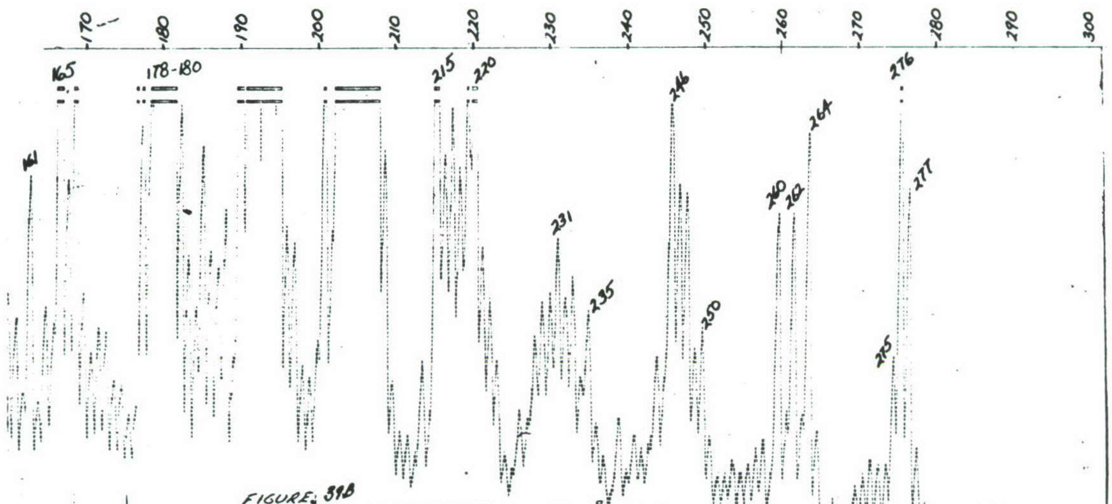


FIGURE 39B
1/2-HR. URINARY EXTRACT
3×10^{-9} GAIN
25 sec post-injection

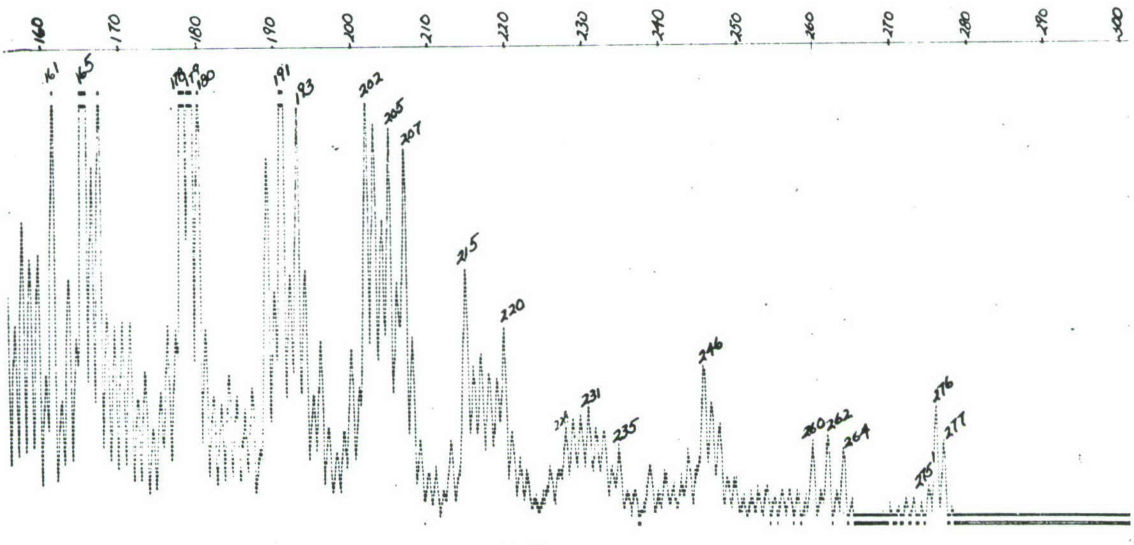


FIGURE 39C
1/2-HR. URINARY EXTRACT
3×10^{-9} GAIN
50 sec post-injection

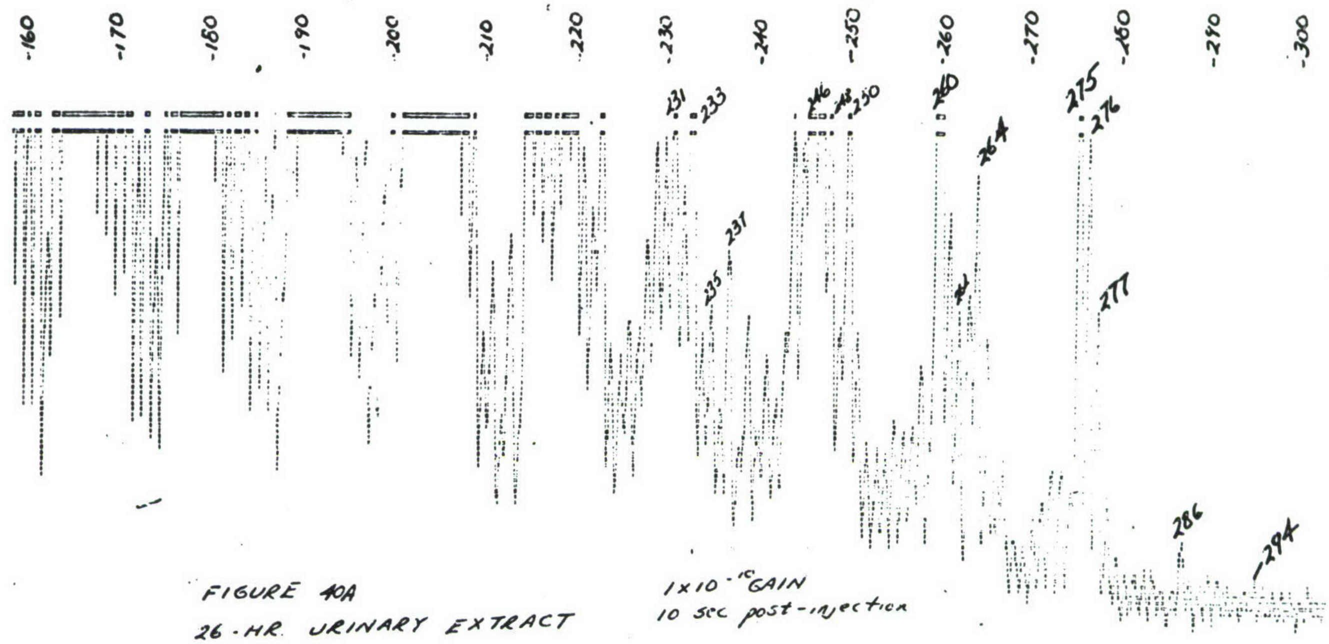


FIGURE 40A
26-HR URINARY EXTRACT
1×10^{-10} GAIN
10 sec post-injection

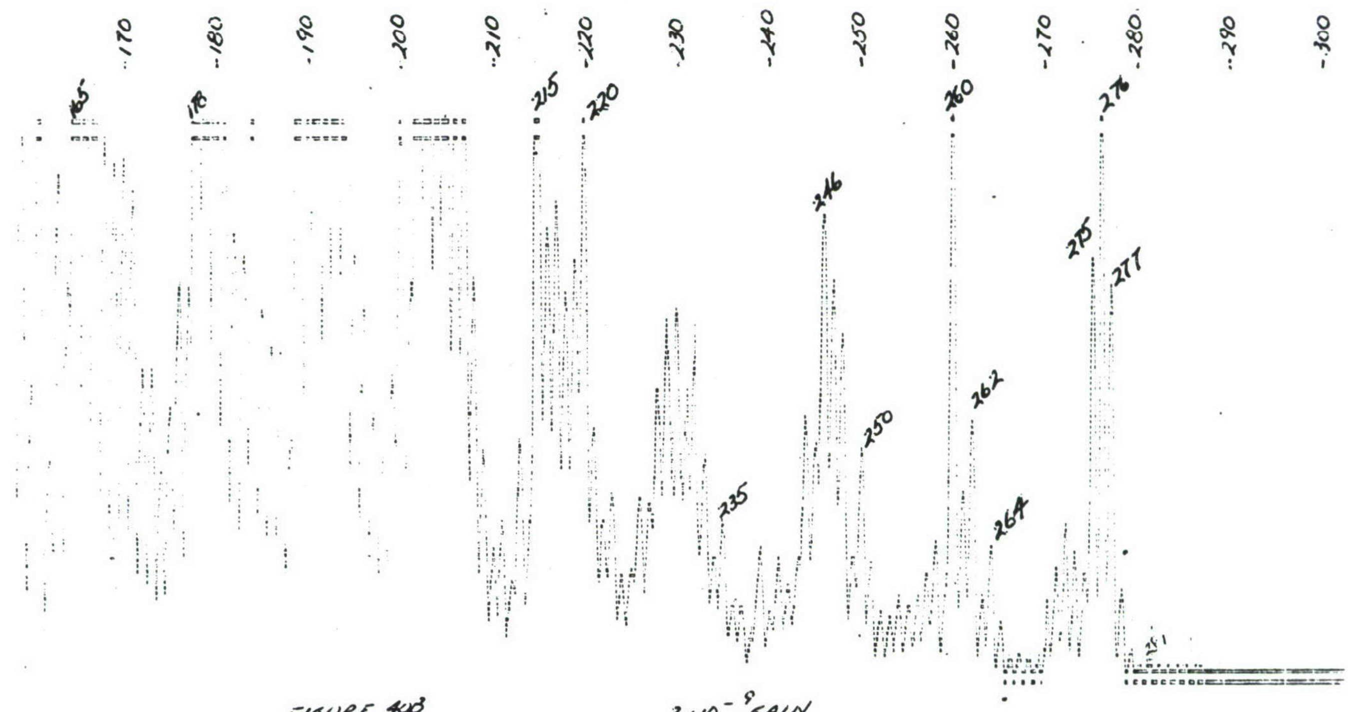


FIGURE 40B
26-HR URINARY EXTRACT
3×10^{-9} GAIN
25 sec post-injection

X. Detection of Amphetamine Use

Previous work at Varian demonstrated submicrogram sensitivity to methamphetamine from aqueous solution.

Amphetamines are volatile, low molecular weight compounds, in contrast to the other common abuse drugs (i.e., barbiturates, narcotics, phenothiazines). Amphetamine mass spectra are characterized by weak molecular ions and abundant low mass ions (see Fig. 41). Thus, in CVA mass spectrometric analysis, amphetamines will suffer interference in this low mass region from ions due to normal body fluid constituents.

Amphetamine volatility suggested head space analysis of a warmed (80^{o}C water bath) urine sample in a manner analogous to the ethchlorvynol detection of section V(4). However analysis of a 1 mg % spiked methamphetamine urine failed to produce a significant increase in ion abundance at m/e 58, 91, 134 relative to the urine blank. This procedure could conceivably be improved by:

(1) heating the urine at a higher temperature.

(2) extracting the drug from urine at pH 11 into a lesser volume of organic solvent (i.e., chloroform), thus concentrating the sample (vapor pressure of a solute is proportional to its mole fraction in solution).

A more promising alternative would involve silylation of a chloroform extract of the body fluid.

MW 149 MW 221

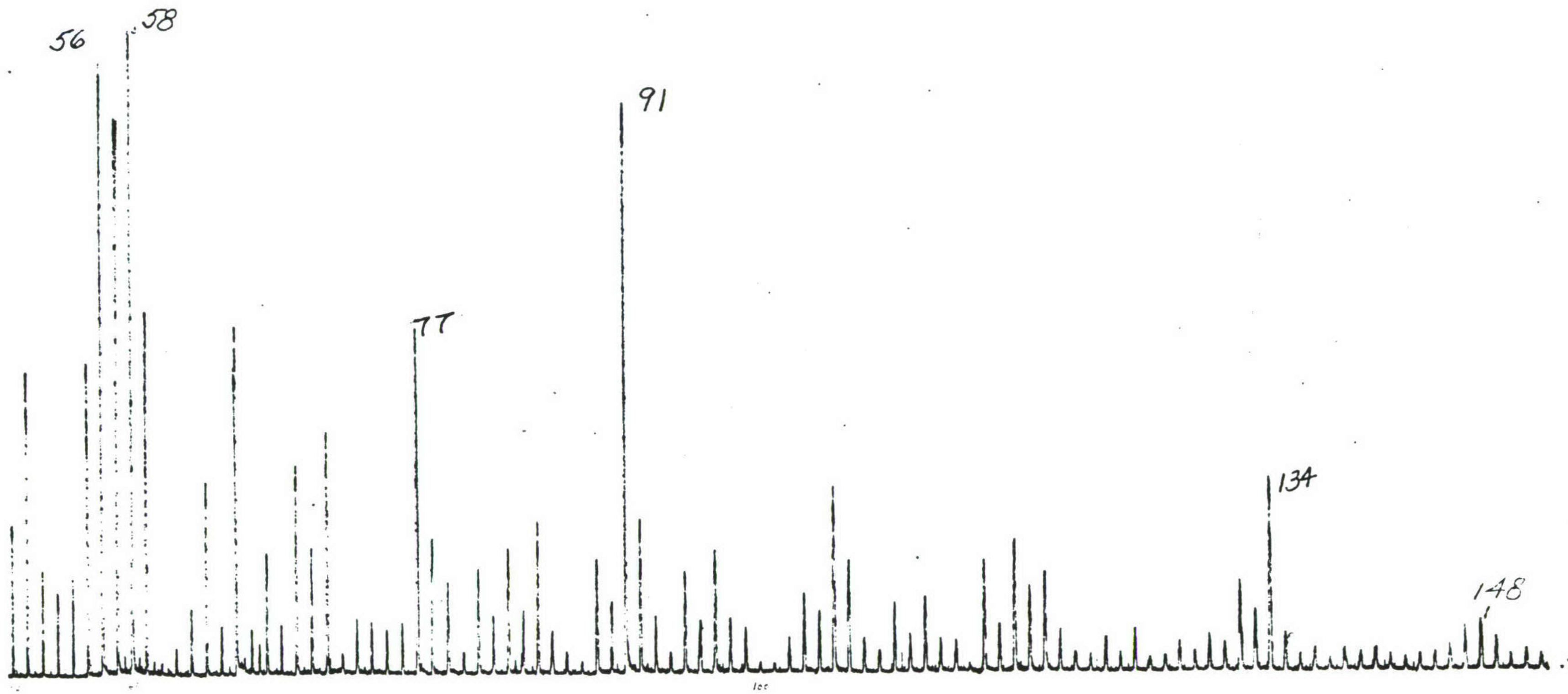
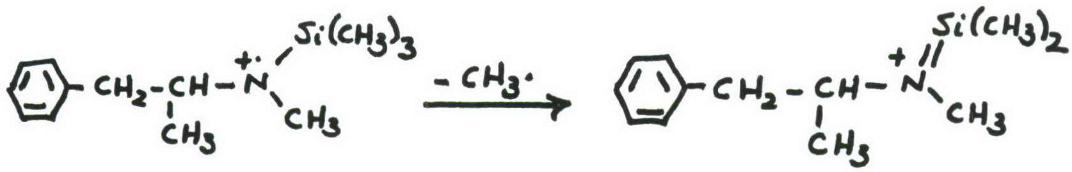


FIGURE 41. CVA MASS SPECTRUM OF METHAMPHETAMINE
m/e 56, 58, 77, 91, 134, 148 - CHARACTERISTIC IONS

One would then monitor the intense $(M-15)^+$ ion of the silyl derivative at m/e 206.



m/e 221 m/e 206

Amphetamine silylation can be achieved in ≤ five minutes, following standard literature procedures.[22]

Adequate time to investigate the silylation study was not available. However this method offers great promise and requires only modest development.

[22] A. H. Beckett et al, J. Pharm. Pharmac. 19, 273 (1967).

XI. Description of the Instrument

The basis of the CVA system (shown schematically in Fig. 42) is a quadrupole mass spectrometer interfaced to a Llewellyn semi-permeable membrane separator. The spectrometer identifies compounds present in a vaporized sample by means of their characteristic mass spectra. Injected samples are vaporized in a silanized stainless steel, heated inlet tube connected to the separator.

The key to Varian's approach to trace analysis is the ability of the Llewellyn separator to interface high vacuum instruments such as the mass spectrometer with vapors at ambient atmospheric pressure by effectively excluding the permanent air gases and high molecular weight compounds while transmitting the medium molecular weight drugs and other moderately condensable elements. This results in an enrichment of the desired sample relative to the diluting components. (This concept is covered by several U.S. patents.)

Transmission of compounds through the membrane separator requires both surface sorption and bulk diffusion. These effects are influenced by the membrane temperature and by the relative chemico-physical properties of the sample compound and the polymeric membrane. The moderate molecular weight, polar compounds typical of abuse drugs and metabolites have high transmission through a dimethylsilicone membrane at elevated temperature. Sensitivity is enhanced by separator design employing multiple membranes with intermediate pumping ports, as shown in Fig. 43. The impedance of the pumping ports is balanced with the impedance of the membrane to the compound being detected. Although a small amount of sample is lost through the pump ports, in a proper design the "undesired" compounds preferentially exit through the pump port and the "desired" compounds preferentially pass through the membrane and into the mass analyzer. In this way, enrichment factors of over one million have been obtained for some compounds.

The quadrupole mass analyzer has several inherent advantages in a drug-screening operation relative to a conventional magnetic mass analyzer. The quadrupole is more portable, relatively inexpensive and requires little experience to maintain and operate. Quality output of the analyzer can be maintained at higher pressures (up to 10^{-4} torr) than magnetic analyzers. The analyzer system can be readily baked at high temperature (i.e., -300°C).

The linear relationship between the rf/dc voltage applied to the quadrupole rods and the mass scale allows direct computer control of the mass analyzer; the computer can direct the analyzer to any arbitrary position in the m/e range of the instrument (as described in Section V). A mass spectrum can be scanned repetitively with dwell times of a millisecond per nominal mass unit, allowing real-time observation of the spectrum on an oscilloscope display.

A photograph of the current instrument is provided in Fig. 44.

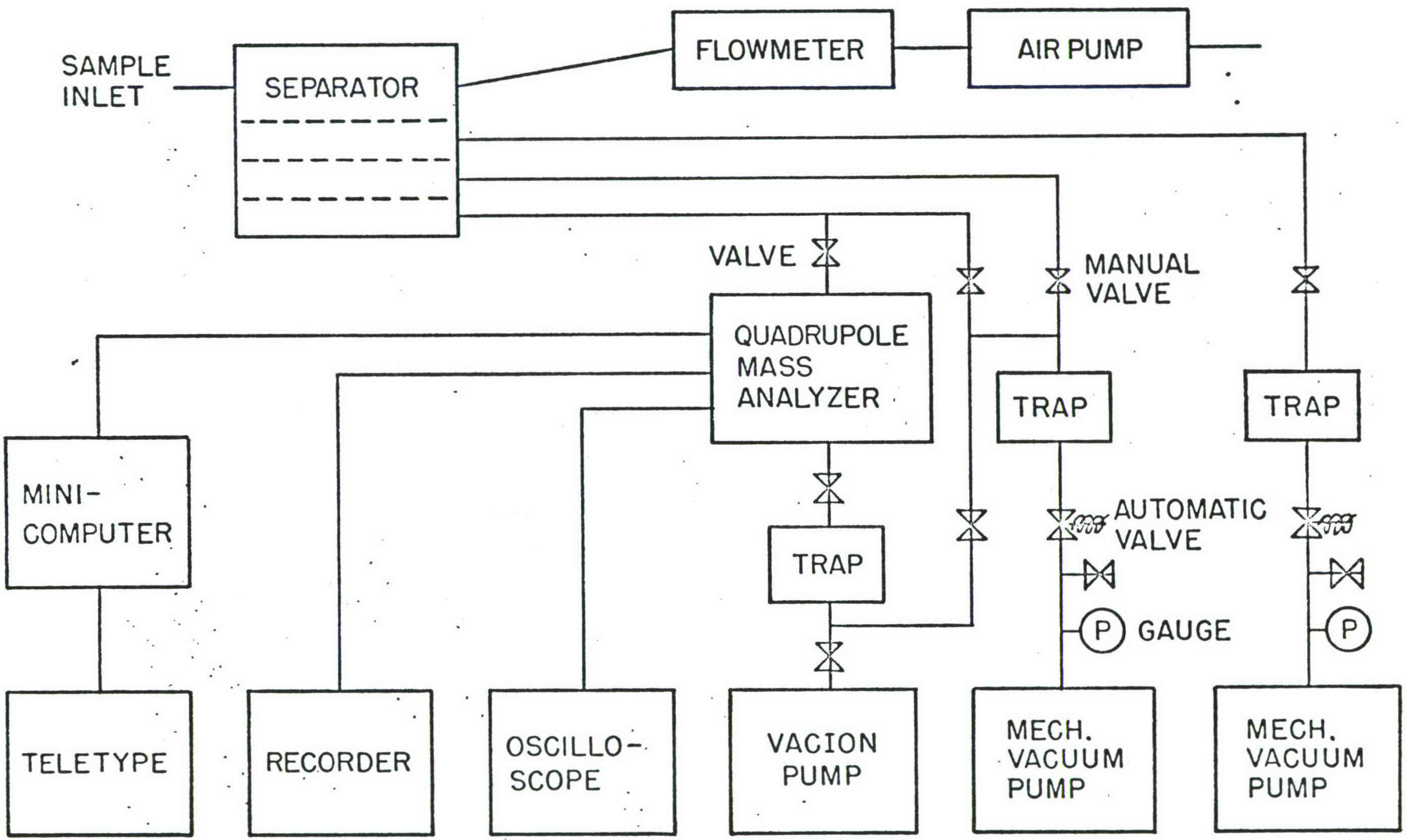


FIGURE 42. CVA-MS SYSTEM SCHEMATIC

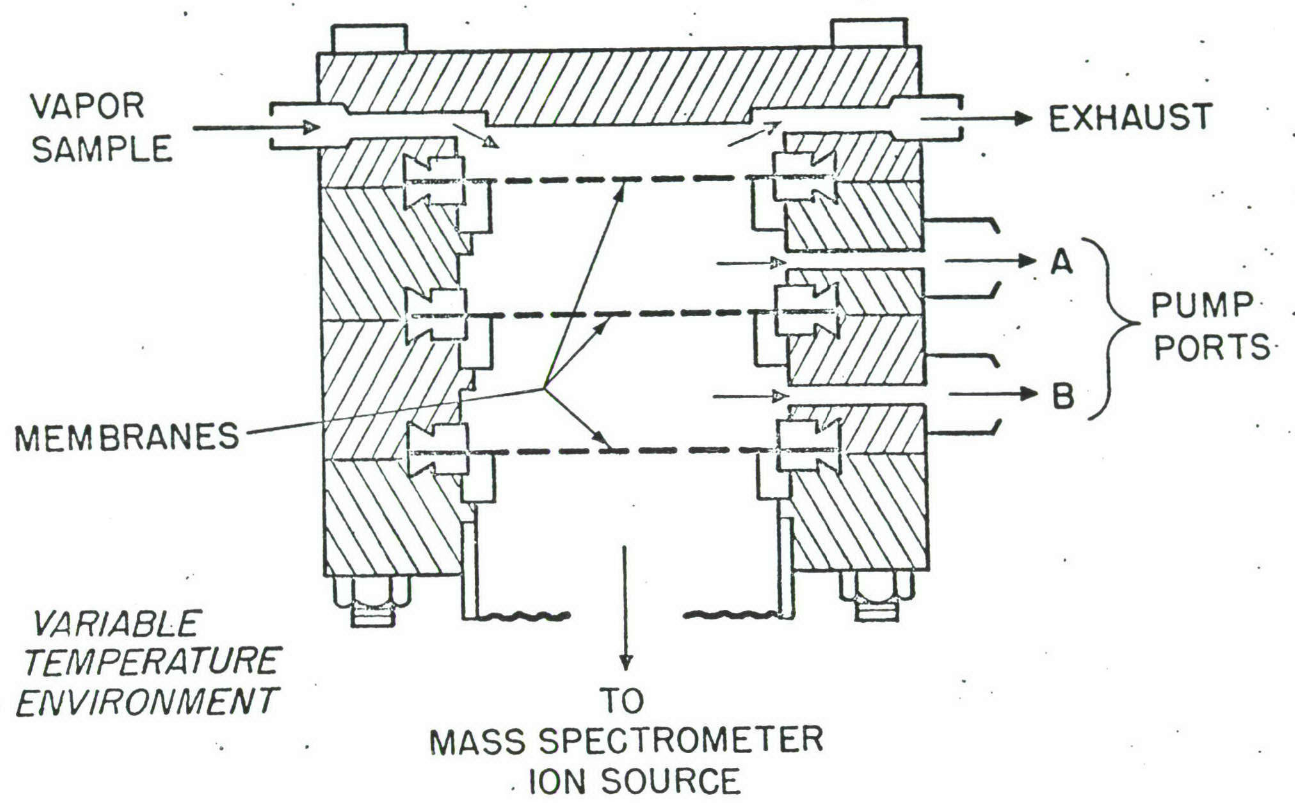


FIGURE 43. MEMBRANE SEPARATOR

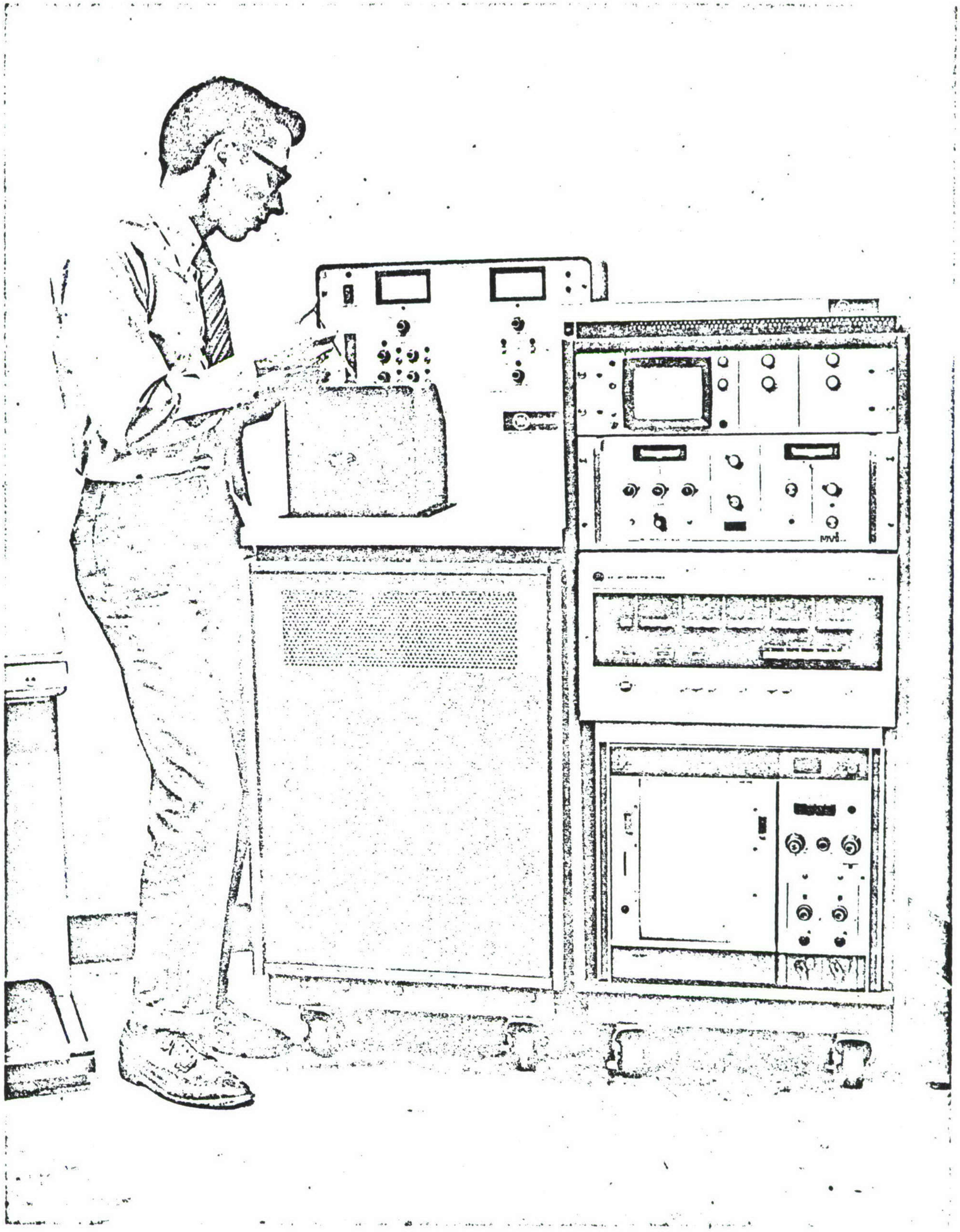


FIGURE 44. VARIAN CHEMICAL VAPOR ANALYSIS-MASS SPECTROMETER (CVA-MS) SYSTEM

XII. Summary and Conclusions

In summary, a range of drugs encompassing barbiturates, tranquilizers, narcotics, sedatives, hallucinogens, antimalarial and antimicrobial agents were selected for CVA-MS analysis. These drugs, representing a wide range of chemico-physical and pharmacological properties, were chosen to demonstrate the universality of the Chemical Vapor Analysis-Mass Spectrometry technique in drug screening, and the feasibility of simultaneous, real-time monitoring for multiple drugs in body fluids.

The selected drugs were successfully analyzed at 100 nanogram-1 microgram sensitivities from standard solutions. Analyses were run at one set of instrument parameters for all drugs. This is a particular advantage of the CVA technique relative to other high volume screening procedures (thin-layer chromatography, gas chromatography, spin and radio-immunoassay, gas chromatography-mass spectrometry).

Body fluid analyses were conducted at Varian Associates and at San Francisco General Hospital. These revealed that urine, blood (Section VII) and gastric fluids were suitable media for drug and metabolite detection. Successful analyses were conducted on both therapeutic and overdose drug level body fluids. Results were confirmed by the hospital toxicology laboratory, using standard methods of analysis. Due to the low volatility and permeability of most drugs at body temperature, detection was not achieved in samples derived from breath, saliva and skin wipings.

The inherent specificity of the mass spectrometric technique relative to chromatographic procedures and current immunoassay procedures was reflected in the absence of false positives encountered in the therapeutic and poisoning case work. This feature is critical in poisoning analysis, where the drug identification is used by the attending physician to prescribe patient therapy. The speed of the technique (five minutes for one sample) should allow the physician to prescribe rational therapy before further patient deterioration.

Initially, direct injection of urine into the CVA-MS system was used extensively; however,further experimentation demonstrated that use of a simple organic solvent extract of the particular body fluid to be analyzed resulted in a considerable reduction of instrument contamination and elapsed

time between sample runs. When used as a mass-screening technique for multiple drugs in urine, utilizing a simple body fluid extract, the CVA-MS technique demonstrated an analysis time of two minutes per sample for a ten percent positive drug population.

Studies of heroin addicts performed at SFGH demonstrated CVA-MS detection of morphine in addict urine three to four days after a fix. The power of the CVA-MS multiple-drug screen capability enabled simultaneous detection of morphine <u>and</u> codeine in the addict urines. Codeine, a previously unreported metabolite of morphine in man, was detected in 80% of the addict urines analyzed. This observation indicates that chronic heroin use induces the O-methylase enzyme system which catalyses the conversion of morphine to codeine and this provides a means with which to differentiate the "hard-core" user from the experimenter.

The CVA-MS drug screen is performed by monitoring the sample for specific ions characteristic of each drug or metabolite. The choice of a quadrupole assembly as the mass analyzer component in the CVA system makes the system amenable to computer operation, as demonstrated in the work at SFGH. A minimal software development would extend computer control to the actual decision-making process, thus lowering the level of technical training required for operating personnel.

Future work should also include: (1) Detection of lysergic acid diethylamide (LSD) and cannabinoids in body fluids. These drugs produce abundant high mass ions which suffer negligible interference from normal body fluid components. A CVA sensitivity of 100 nanograms is predicted. This sensitivity should allow detection of the low body fluid levels observed in use of such drugs. (2) Monitoring of a single mass ion over the full transmission time (one-two minutes) of the particular drug through the membrane, with computer intergration of the area under the signal-time plot. This procedure should allow 10-100 nanogram sensitivities for the abuse drugs in body fluids.

APPENDIX I. REFERENCES ON MASS SPECTRAL FRAGMENTATION OF DRUGS

(1) Barbiturates - R. T. Coutts and R. A. Locock, J. Pharm. Sci., 57, 2096 (1968).

(2) Benzodiazepines (diazepam, oxazepam) - W. Sadée, J. Med. Chem., 13, 475 (1970).

(3) Morphine alkaloids (morphine, codeine) - D. S. Wheeler et al, J. Am. Chem. Soc., 89, 4494 (1967).

(4) Phenothiazines (chlorpromazine) - J. N. T. Gilbert and B. J. Millard, Org. Mass Spec., 2, 17(1969).

(5) Glutethimide and alpha-phenylglutarimide - G. Bohn and C. Rücher, Archiv. f. Tox., 23, 221 (1968).

(6) Chloroquine, cocaine - N. C. Law et al, Clin. Chim. Acta, 32, 221 (1971).

(7) Hallucinogens (i.e., LSD) - S. W. Bellman, J. Assn. Off. An. Chem., 51, 164 (1968).

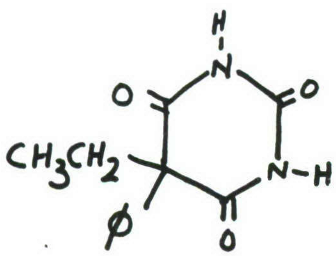
APPENDIX II. GENERIC-COMMERCIAL DRUG NAMES

Generic	Commercial
Phenobarbital	Luminal
Secobarbital	Seconal
Pentobarbital	Nembutal
Diazepam	Valium
Oxazepam	Serax
Morphine	-
Methadone	Dolophine
Codeine	-
Cocaine	-
Glutethimide	Doriden
Chloroquine	Nivaquine
Sulfamethazine	Diazil
Chlorpromazine	Thorazine
Trimethoxyphenethylamine	Mescaline

APPENDIX III. METABOLIC PATHWAYS OF DRUGS STUDIED

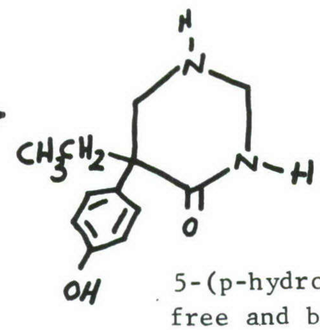
NOTE: Pathways for morphine, methadone are discussed in the text, pages 76, 84-85 respectively.

A. PHENOBARBITAL



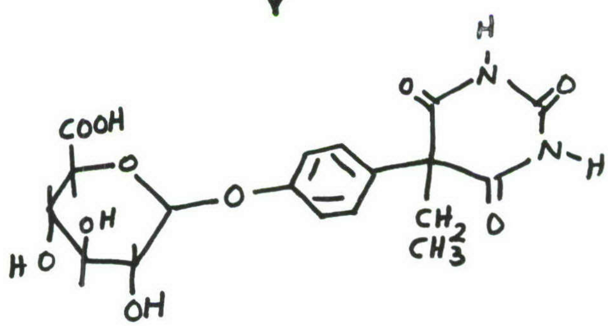
Phenobarbital
50% in urine

hydroxylation



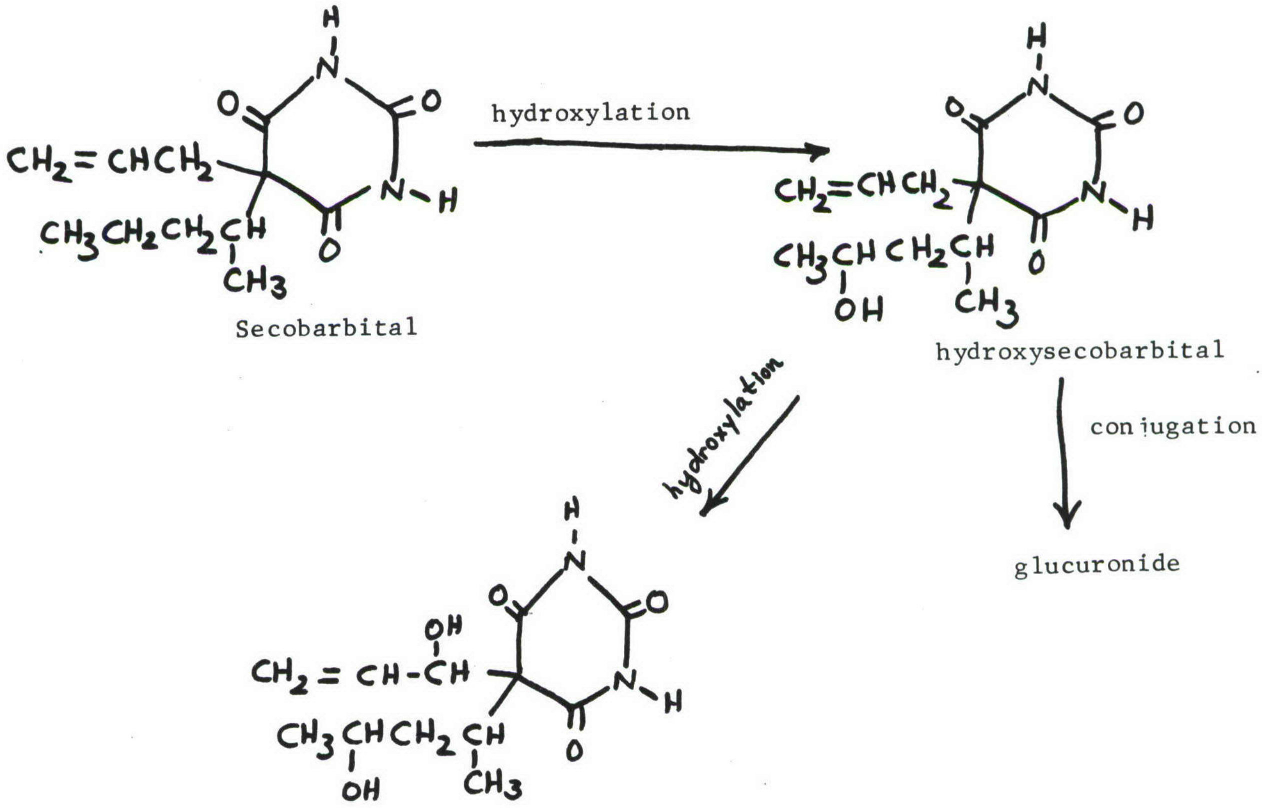
5-(p-hydroxy)phenobarbital
free and bound - 50% in urine

conjugation



hydroxyphenobarbital glucuronide

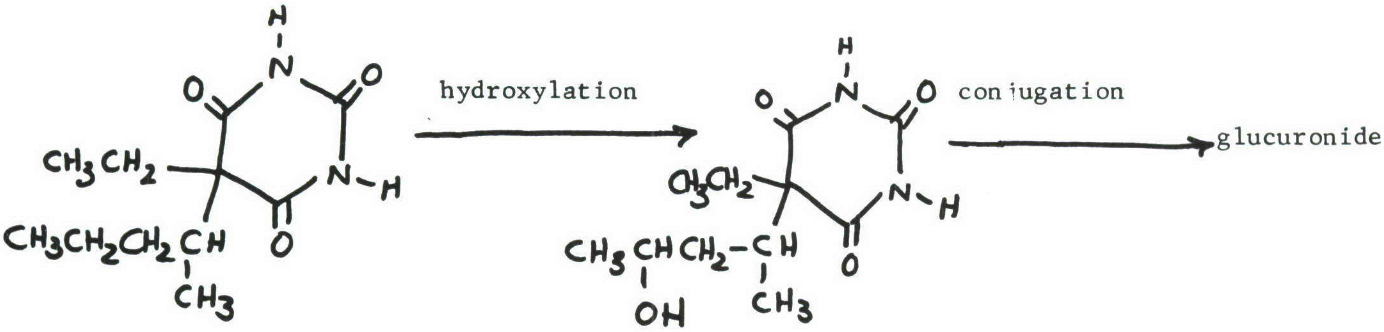
B. SECOBARBITAL



Secodiol

Major metabolite and major species in urine

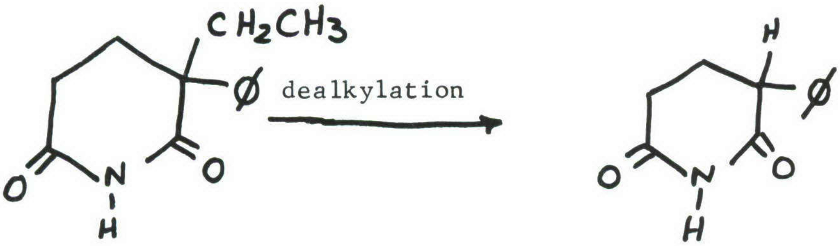
C. PENTOBARBITAL



Pentobarbital

Hydroxypentobarbital
Major metabolite and
major species in urine

D. GLUTETHIMIDE

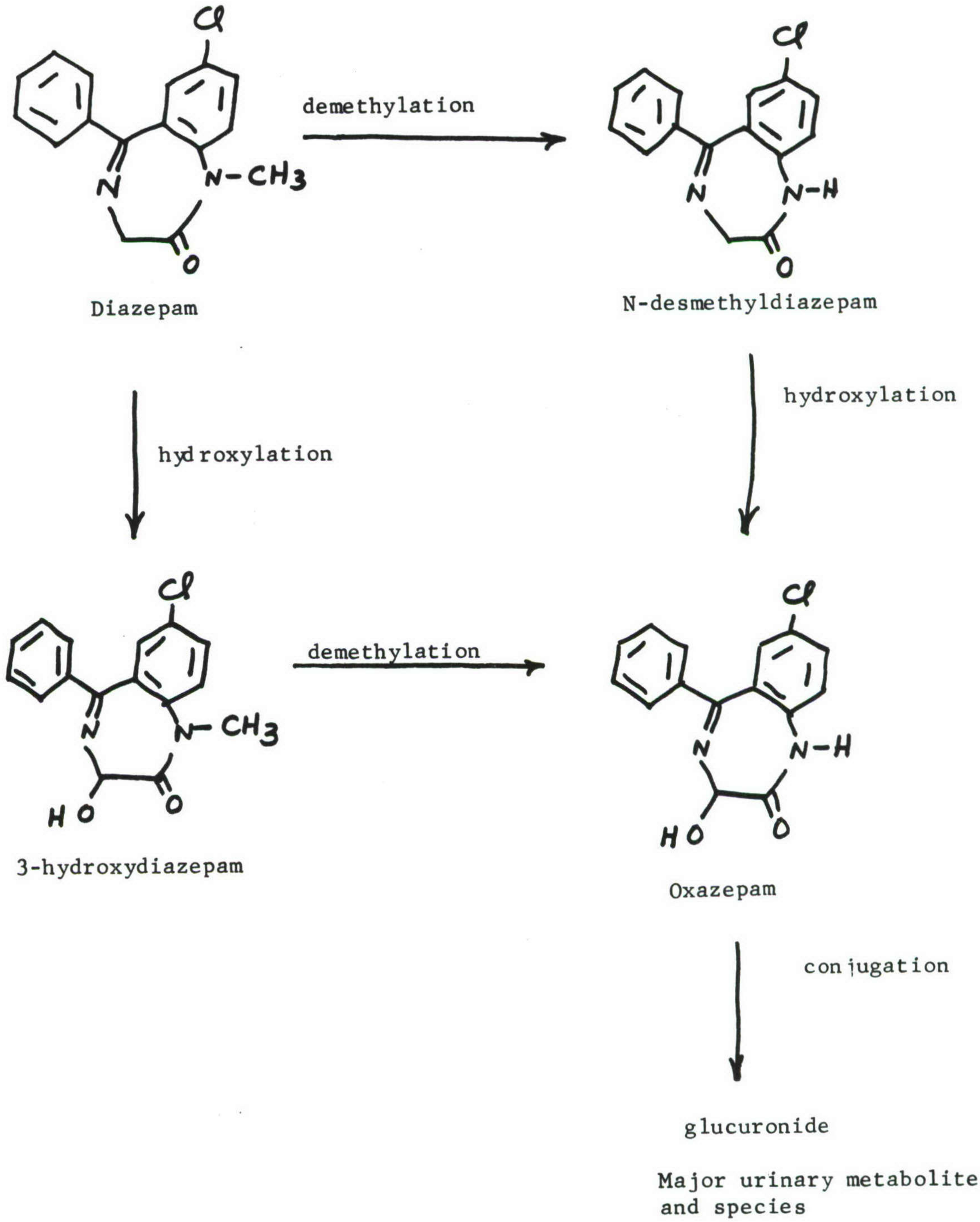


Glutethimide

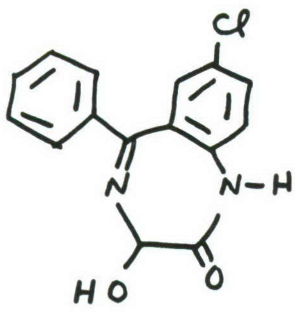
Alpha-phenylglutarimide

Major metabolite and major
species in urine

E. DIAZEPAM


Diazepam
demethylation
N-desmethyldiazepam
hydroxylation
hydroxylation
3-hydroxydiazepam
demethylation
Oxazepam
conjugation
glucuronide
Major urinary metabolite
and species

F. OXAZEPAM



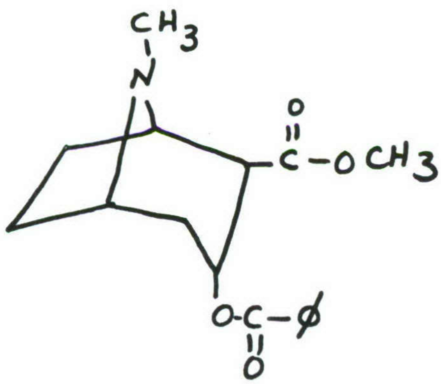
Oxazepam

conjugation →

glucuronide

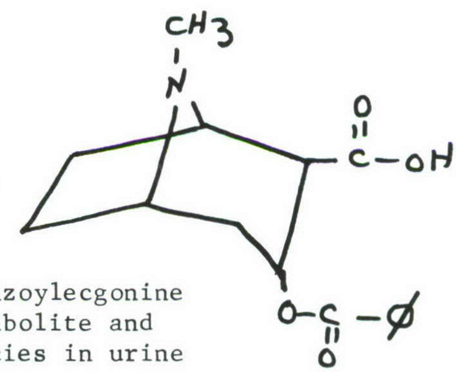
Major metabolite and major species in urine

G. COCAINE

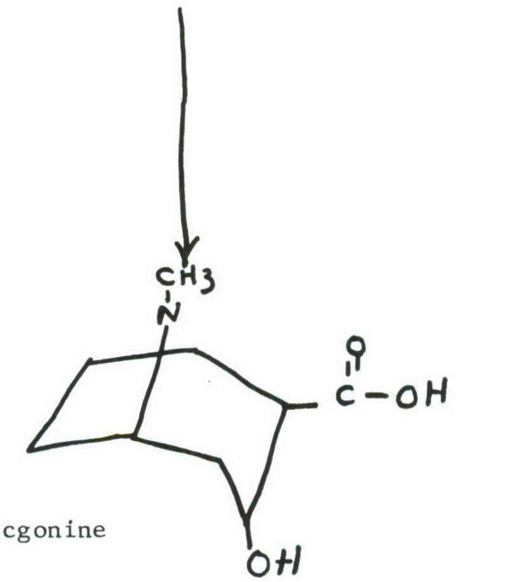


Cocaine

demethylation →



Benzoylecgonine
Major metabolite and major species in urine

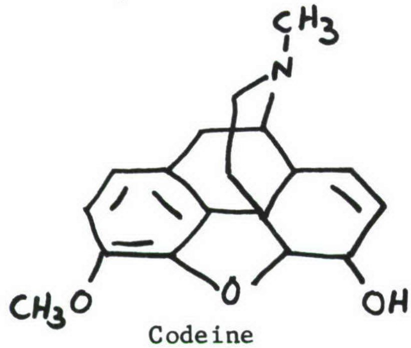


Ecgonine

← conjugation

glucuronide

H. CODEINE

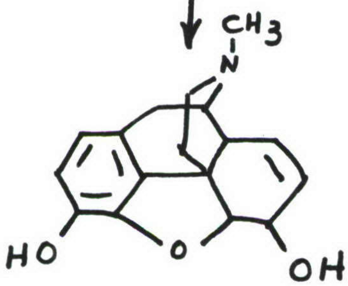


Codeine

conjugation → glucuronide

Major metabolite and major species in urine

O-demethylation ↓

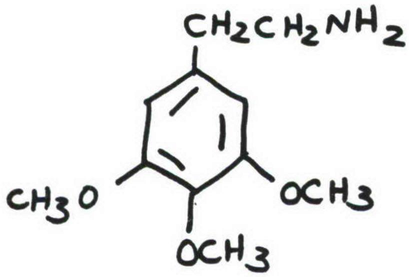


Morphine

conjugation → glucuronide

Present at ca. 20% relative to codeine glucuronide

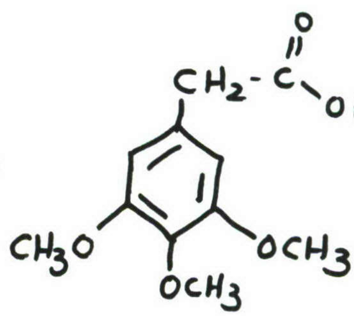
I. MESCALINE



Mescaline

(3,4,5-trimethoxyphenethylamine

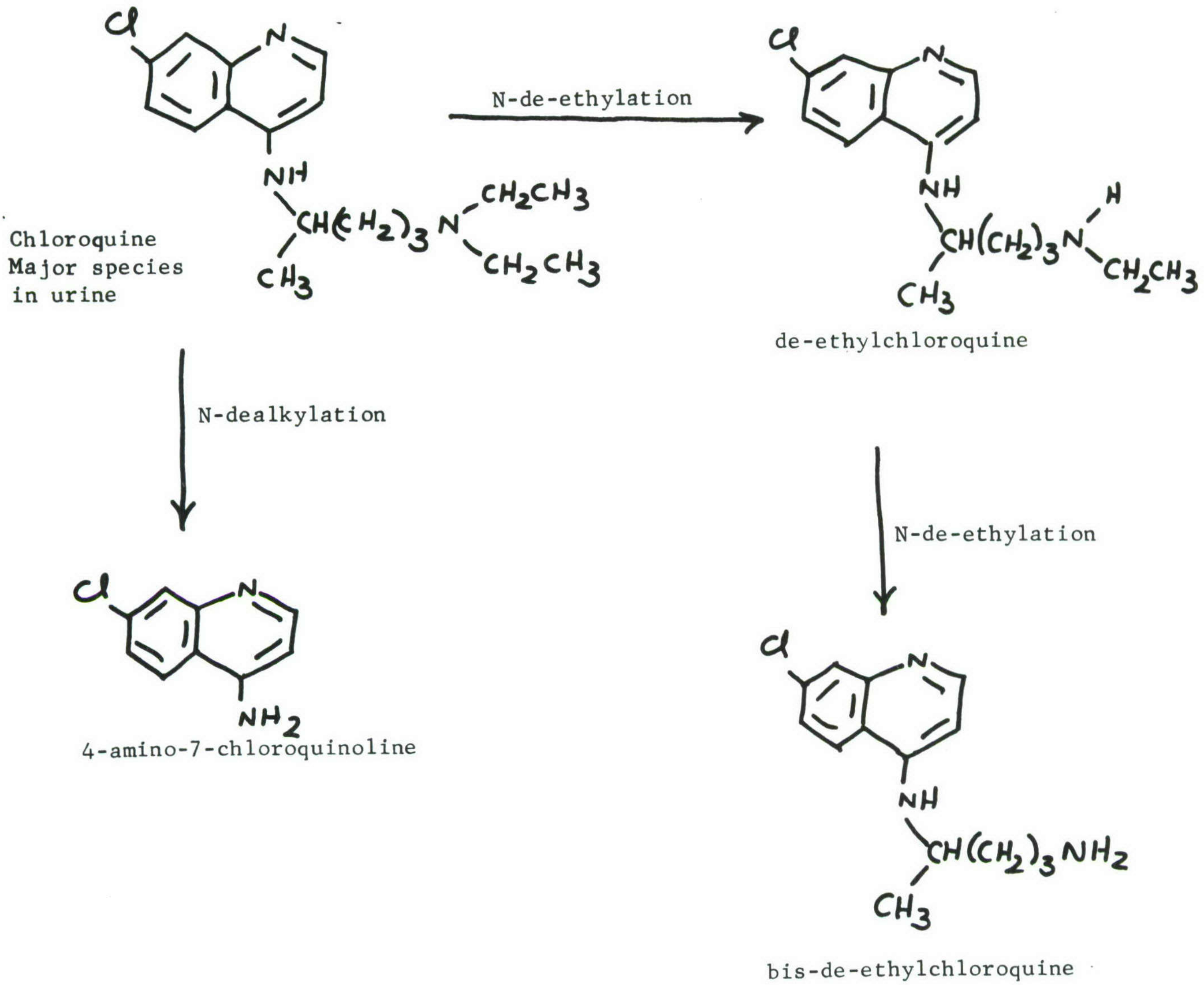
→



3,4,5-trimethoxyphenylacetic acid

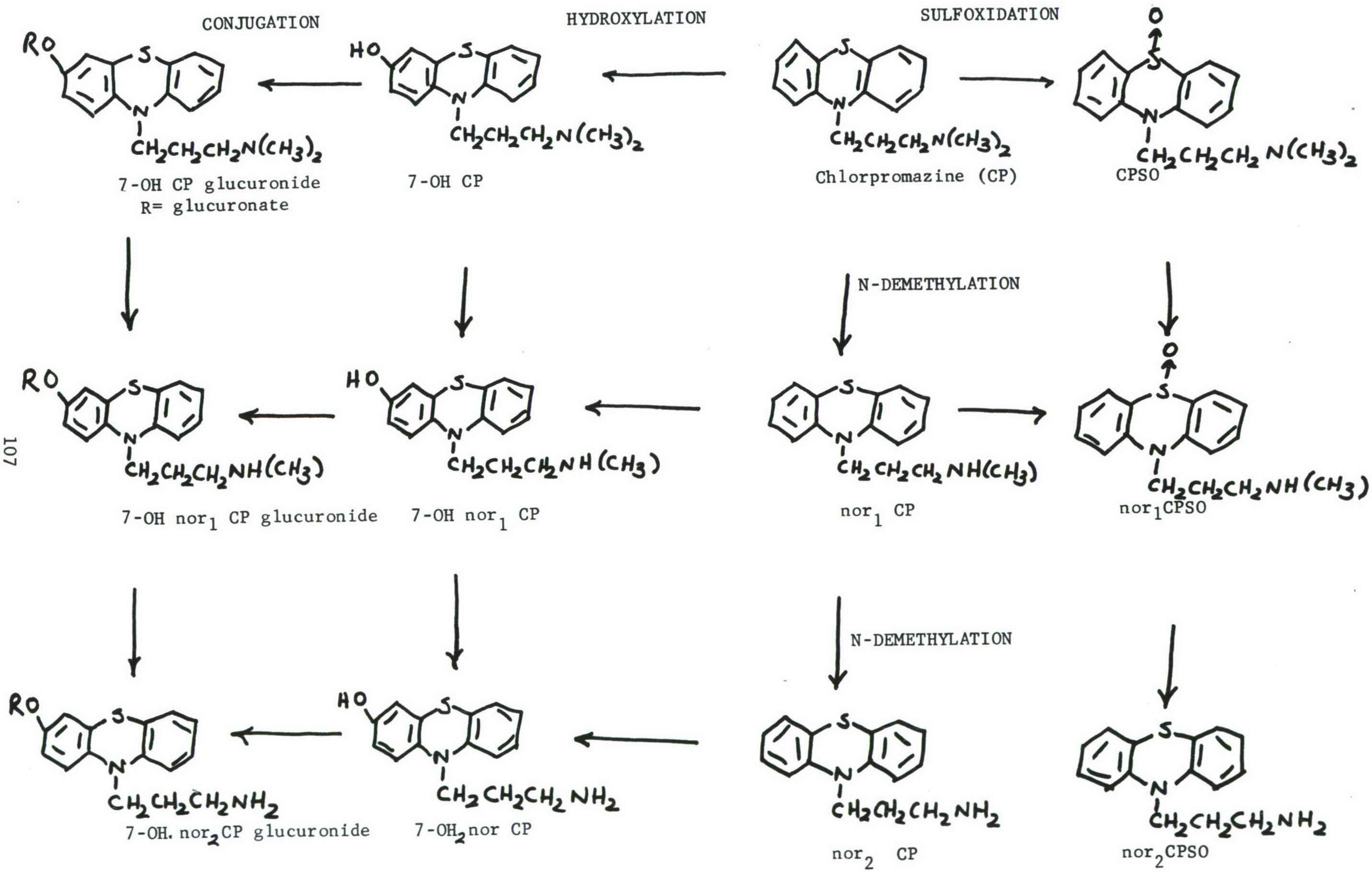
Major metabolite and major species in urine

J. CHLOROQUINE



K. CHLORPROMAZINE

Major urinary metabolites and species are sulfoxides and glucuronides



APPENDIX IV

Procedure 1 Preparation of Saliva Sample for Drug Analysis

(1) The saliva is diluted with distilled water (1 ml saliva + $\sim$4 ml water) and filtered on an ultrafiltration apparatus utilizing PM-10 membranes (cut off $\sim$ Molecular Weight 10,000 will exclude the salivary hydrolytic enzymes and mucoprotein).

(2) The filtrate is adjusted to the appropriate pH (to convert the drug to be detected to its neutral, organic-soluble form) and extracted 1:1 with chloroform or ether. The organic phase is evaporated down to $\sim$ 50 µl and injected into the CVA instrument.

Procedure 2 Collection and Preparation of Skin Wipe Sample for Drug Analysis

(1) A cotton swab is dipped repeatedly in acetone and used to wipe twice the area between the forearm and wrist ($\sim$10 in^2) on the inside of the arm. After each of the repeated wipes ($\sim$ 1 in^2 each) over parts of this area, the swab is dipped in a collection beaker of 5 ml acetone.

(2) The acetone is evaporated down to 50 µl and injected.

Procedure 3 Collection and Preparation of Breath Sample for Drug Analysis

(1) The subject is required to breathe for five minutes into a gas bubbler tube immersed in a beaker of ice.

(2) The tube condensate is dissolved in $\sim$1 ml distilled water.

(3) The aqueous solution is adjusted to the appropriate pH and extracted into ether (1:1). The ether phase is evaporated to $\sim$100 μl and 50 μl is injected into the spectrometer.

Procedure 4 Urine Analysis

(1) Adjust urine to desired pH (pH2 for analysis for acidic drugs, pH9-10 for basic drugs), with concentrated hydrochloric acid or ammonium hydroxide.

(2) Extract 4:1 with desired organic solvent (ether for acidic extract, 4:1 chloroform: isopropanol for basic extract). Typically extract 4 ml → 1 ml.

NOTE: If urine is collected by catheterization, centrifuge for five minutes prior to work-up, to remove epithelial cells.

(3) Inject 10 μl extract. If no drug positive is observed, inject 50 μl, 250 μl until drug positive is observed.

Procedure 5 Blood Analysis

(1) Centrifuge sample for five minutes. Pour off supernate (plasma).

(2) Work up plasma as in case of urine (above). Extract 1 ml → 250 μl.

Procedure 6 Gastric Content Analysis

(1) Work up sample as in case of urine. Centrifugation was not necessary in our experience.

(2) Inject 5 μl initially. Initial injection of a larger sample can overpressure the analyzer due to the extremely high drug level encountered in gastric contents.

SUPPLEMENTARY REPORT

ANALYSIS OF MORPHINE IN URINE BY CVA-MASS SPECTROMETRY

Report for Supplement

to

Contract DAAD05-70-C-0197

by

Seth R. Abbott
James T. Arnold
Kay O. Loeffler

Varian Associates
Palo Alto, California 94303

February 1974

APPROVED FOR PUBLIC RELEASE; DISTRIBUTION UNLIMITED

US ARMY LAND WARFARE LABORATORY

ABERDEEN PROVING GROUND, MARYLAND 21005

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

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FOREWORD

This report is a supplement to the previous report titled, Analysis of Drugs and Drug Metabolites in Body Fluids by CVA-Mass Spectrometry. Upon completion of that effort it was decided that further work would be productive in the area of morphine detection since that was the drug of prime interest. This effort concentrated on three areas: (1) a determination of the absolute sensitivity of the system for morphine, (2) ways of increasing the system sensitivity, and (3) the effect of sample storage conditions on sample concentration. This work was under the technical supervision of H. Clay McDowell of the Applied Physics Branch, US Army Land Warfare Laboratory. This project was designated as Task 02-P-72, Metabolized Drug Detection.

I. PURPOSE OF PROJECT

DETERMINE CVA CAPABILITY FOR DETECTION OF HEROIN ABUSE

Heroin is excreted in the urine of humans largely as free and conjugated morphine. Fifty to 90% of the dose is excreted in urine as bound morphine (morphine glucuronide) and 1-15% as free morphine[1]. Fifty percent of the urinary excretion occurs within eight hours and 90% within 24 hours[2,3]. Assuming a 50% of dose urinary excretion, a 200 milligram per day average dose and two liters per day average addict urinary output, 24-hour morphine level in addict urine will be 45 micrograms per milliliter bound and one to five micrograms per milliliter free morphine.

Studies at San Francisco General Hospital indicated urinary bound morphine levels of 250 to 500 nanograms per milliliter in two of eight addict urines collected three to four days after admission to a detoxification program[4]. These levels were down by two orders of magnitude relative to admission levels.

[1] In one study, a higher level of urinary excretion of morphine dose was observed in an addict (98%) than in a group of non-addicts (58-84%). A. Stollman and C. P. Stewart, "Toxicology: Mechanisms and Analytical Methods", 1960; E. L. May and T. K. Adler, "The Biological Disposition of Morphine and its Surrogates", World Health Organization, Geneva 1962.

[2] L. S. Goodman and A. Gilman, The Pharmacological Basis of Therapeutics, (The MacMillan Company, New York, 1970).

[3] P. Paerregard, Acta Pharmacol. (Kobanhaven), 14, 53 (1957).

[4] Private communication by Mr. Udo Boerner, San Francisco Department of Public Health, San Francisco General Hospital.

Previous CVA studies indicated sensitivity to morphine of approximately one microgram. Analyses were performed on extracts of two milliliters urine, corresponding to detection of 500 nanograms/milliliter morphine in urine. This capability allowed morphine detection in both hydrolyzed and unhydrolyzed urines collected upon admission, and occasional detection of trace morphine in hydrolyzed urines collected three to four days after dose.

The limiting factors in CVA morphine detection were believed to be adsorption of morphine onto the inlet and separator stainless steel surfaces and low permeability of the polar morphine in the nonpolar dimethyl silicone membranes.

The purpose of the current study was to extend CVA sensitivity to detection of 100 nanograms/milliliter morphine in urine in order to allow detection of heroin abuse three to four days after dose. Derivatization of morphine to a less polar form, in order to reduce adsorption onto heated inlet and separator surfaces and increase membrane permeability, was proposed. Use of a glass inlet was proposed in the expectation that glass was more amenable to chemical deactivation of the surface area than stainless steel.

Storage of dilute samples was investigated. Containers possess a certain "active site potential" for adsorption of polar molecules. At high sample levels (greater than 10 micrograms/milliliter) the number of active sites is expected to be much less than the number of sample molecules. However at lower levels, a point could conceivably be reached at which the active surface sites adsorb an appreciable percentage of the sample.

II. DERIVATIZATION

Replacement of active hydrogen in a molecule by a nonpolar group such as a trimethylsilyl (TMS) or a methyl group reduces molecular polarity and decreases the possibility of intra- and intermolecular hydrogen bonding. Where there is marked intermolecular hydrogen bonding in the parent molecule, the derivative is markedly more volatile. The derivative should also have increased permeability in nonpolar dimethyl silicone membranes. Stability of the molecule is enhanced by derivatization due to reduction in the number of reactive sites with active hydrogen. Thus surface adsorption is reduced.

The polarity of morphine is due predominantly to the phenolic hydroxyl group.

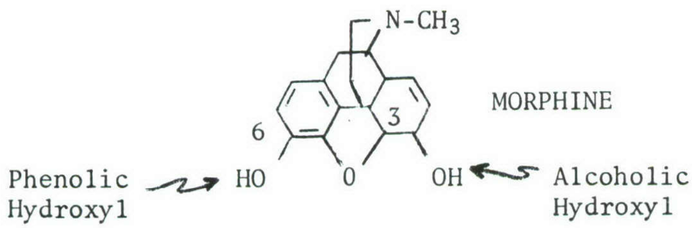


FIGURE 1. STRUCTURE OF MORPHINE

Derivative characteristics of interest in morphine detection by the CVA technique are:

1) Polarity -- polarity of the parent molecule must be reduced to increase volatility, reduce surface adsorption, increase membrane permeability.

2) Nature of mass spectrum in CVA analysis -- interference from endogenous components of urine or plasma is severe at low mass (below m/e 100) and drops off sharply with increased mass. Interference at m/e 200 is equivalent to approximately 100 nanograms/milliliter of a typical abuse drug whereas interference at m/e 300 is equivalent to approximately 10 nanograms/milliliter. One expects continued reduction in interference with higher mass (based on mass fragmentography studies in the literature). The upper mass limit of the current CVA quadrupole mass analyzer is m/e 350. Thus, it is a requirement that the derivative produce high intensity ions in the m/e 250-350 region.

3) Ease of derivatization -- the preferred reagent should derivatize morphine to a single product in the heated CVA inlet, obviating external preparation. The reagent should be commercially available, inexpensive and should not constitute a storage problem.

Although derivatization increases molecular weight, experience with molecules of molecular weight up to 600 indicates that sample polarity is a far more significant factor in membrane permeability than molecular weight.

Morphine derivatization in gas chromatographic analysis has historically utilized acetylation[5] and silylation[6]. Less common methods are trifluoroacetylation[7] and methylation[8].

Acetylation and trifluoroacetylation are rapid reactions amenable to on-column derivatization in gas chromatography. However, multiple products are formed (i.e., mixture of 6-monoacetylmorphine and 3,6-diacetylmorphine). In addition the reagents, acetic anhydride and trifluoroacetic anhydride, are highly reactive towards water and constitute a storage problem. For these reasons, this study focused on silylation and methylation.

Silylation of morphine was not amenable to inlet derivatization. Derivatization was achieved by external preparation; i.e., a thousand-fold excess (relative to morphine level) of bis-trimethylsilyl acetamide (BSA) was added to an extract of two milliliters urine. The solution was heated for five minutes at 60°C to effect reaction. A literature mass spectrum of bis-(O-trimethylsilyl) morphine is appended (Fig. 2). The base peak of the mass spectrum is the molecular ion at m/e 429 (14%Σ). However the quadrupole mass limit was m/e 350. The most intense ion in the m/e 250-350 region is that at m/e 287 (1%Σ). Due to the weakness of the m/e 287 ion and the need for external derivatization, attention was focused on methylation.

Methylation is a rapid means of derivatizing phenolic hydroxyl groups, amenable to inlet derivatization in gas chromatography. Methylation should produce a single product, 6-methylmorphine (codeine) due to much

[5] S. J. Mulé, Anal. Chem., 36, 1907 (1964).
[6] K. D. Parker *et al*, J. Forensic Sci., 10, 17 (1970).
[7] W. O. Ebbighausen *et al*, J. Pharm. Sci., 62, 146 (1973).
[8] E. Brochmann-Hanssen and T. O. Oke, J. Pharm. Sci., 58, 370 (1971).

higher reactivity with the phenolic hydroxyl than with the alcoholic hydroxyl of morphine[8]. The methylating reagent, Methelute™, is 0.2 molar trimethylanilinium hydroxide (TMAH) in methanol and is commercially available and inexpensive (Pierce Chemical Company). The reagent is stable to water and can be stored under room temperature and room light conditions.

III. EXPERIMENTAL CONDITIONS

A glass inlet was substituted for the original stainless steel inlet. Glass is more readily silanized than stainless steel and is less reactive towards organic molecules at high temperature.

On the supposition that glass is a more suitable material than stainless steel from the standpoint of the number of active sites which might trap some of the sample, a glass inlet was designed and constructed. The design was directed toward three specific requirements; namely, (1) no free path for liquid to reach the separator, (2) sufficient volume to contain all the flash vaporized sample injected, and (3) facility for maintaining temperatures of up to 300°C. Figure 3 shows the glass arrangement. The volume of the bulb is approximately 25 cm^3 which is the approximate expansion volume of 25 microliters of sample material usually injected. The inverted "J" exit port ensures exclusion of liquid drops from the separator. The entire glass inlet is enclosed in aluminum which can be heated electrically to a controlled temperature, and the assembly is housed in a small oven to help maintain uniform temperature and reduce heat loss.

Most of the analyses were carried out without benefit of computer. On an experiment of the scale undertaken, manual reduction of data was deemed more economical than a major software revision which would have involved considerable manipulation of output formats without adding notably to the information in the results.

The programming was reviewed to determine whether data smoothing would improve the sensitivity of the results. The original program algorithm involved basically the following steps:

1) Accumulation of intensity values for selected peaks after injection of a blank sample until each peak had reached its maximum or crest value.

2) Storing the crest intensity values for the selected peaks due to the blank sample.

3) Accumulation of intensity values as in (1) for a "live" sample.

4) Storing the crest intensity values for the live sample as in (2).

5) Subtraction of the crest values in the blank sample from the crest values in the live samples.

6) Comparison of the differences from (5) with predetermined threshold levels.

7) Printing the results.

A visual scan of the printed results identifies the detection of materials characterized by the selected mass peaks.

One modification of the program allows the screening of a sample for a number of drugs by the appropriate selection of twenty peaks (more

or fewer could be chosen) and subsequent peak matching with spectral characteristics of drugs from a preselected list. The program uses the same crest comparison scheme as outlined above. The printout includes the names of the materials detected and the numerical increments of peaks if desired.

The detection algorithm itself might be improved; however, no major gains can be predicted with high confidence due to the variability of the surge in all peaks occurring with injection.[9] Time averaging could certainly reduce the fluctuation of the crest values and lower the false alarm rate or increase the sensitivity by a small factor. One routine which was developed was simply to integrate the incoming peak values for a specified time (the cresting time). This method appeared to show some improvement, but it requires careful determination of the starting and cresting times.

[9]Two developments since the computer program work was concluded are notable in this section:

First, with the development of a new separator, the surge following injection was substantially reduced. In view of this fact, the uncertainty of peak values and peak differences was much less, and more sophisticated processing of data is likely to be quite productive.

Second, the manual analysis of data in the experiments has demonstrated that in the entire high mass region accessible (m/e = 200 to m/e = 350) and probably at higher mass, the background peaks of urine samples are uniformly quite low and fairly constant, allowing for the difference between odd and even peaks. This fact allows a utilization of non-drug peaks to construct a background value to be subtracted from a drug peak, thereby reducing the requirement for injection of blank samples. The data handling described in Section IV outlines a method which could easily be assigned to the computer to generate more sensitive detections and to identify all anomalous peaks in a selected set scanned.

The spectrometer has been treated as a nominal mass device and the control programs have been based on directing the quadrupole to the nominal mass. If at high mass there is as much as 1/4th of a mass deviation from nominal mass, the results will be less favorable. The control program was modified to permit setting to 0.12 a.m.u. in the mistaken view that fragment ion values would be fractional. (Actually this correction is not necessary as was later discovered.)

The inlet-separator system was silanized periodically (ten microliters every fifth sample was sufficient) to deactivate the glass and stainless steel surfaces. Since water can desilanize heated surfaces by hydrolysis of silyl groups, helium was used as an inlet carrier gas rather than air, which contains moisture.

Two sets of urine samples, spiked at levels of 10, 30, 100, 300, 1000 nanograms/milliliter morphine, and refrigerated for two weeks in polypropylene and untreated glass bottles respectively were supplied in person by Mr. Clay McDowell and Dr. Martin Lonky, who witnessed subsequent sample preparation and analysis.

An additional set of samples of Varian donor urine were spiked at 100, 500 and 1000 nanograms/milliliter and stored in untreated glass bottles under room temperature, room light conditions for one month prior to analysis.

A literature study of extraction of morphine from plasma indicated a 20% increase in recovery upon use of silanized glassware versus normal glassware (increased from 65 to 85% recovery)[10]. The use of polypropylene storage bottles was investigated in expectation that a polypropylene surface contains less active sites for adsorption than a glass surface (see Section IV, B).

Sample preparation was as follows: Ten milliliters urine was adjusted to pH 9.3 with concentrated ammonium hydroxide and extracted with 20 milliliters of 4:1 chloroform-isopropyl alcohol. The organic phase was evaporated to dryness and the residue taken up in 125 microliters acetone. Five microliters Methelute™ was drawn up into a 50-microliter syringe, followed by 25 microliters of urine extract-acetone solution. The syringe (representing the extract of two milliliters urine) was then injected into the CVA inlet.

CVA conditions were membrane temperature 180°C, inlet temperature 275°C, analyzer temperature 100°C. The analyzer was not run at the maximum rated temperature of 185°C due to increased thermionic emission from the first dynode of the electron multiplier at elevated temperature.

[10] A. E. TaKemori, Biochem. Pharmacol. 17, 1627 (1968).

IV. RESULTS

A. Army Urines -- Glass Bottles.

The mass spectra of the methylated Army urine extracts are appended in Figures 4A-D.

The m/e 296-7 and 301-7 ions are due to endogenous urinary components. An average intensity and 95% (2σ) confidence limits are calculated over this region, yielding a regional urinary background. The m/e 298 ion is assumed to belong to the regional population in the absence of codeine. Codeine will produce a strong m/e 298 ion intensity (M-1 ion, base peak) if present in the sample.

A check of the assumption that m/e 298 belongs to the regional population was possible: An Army donor "blank" urine[11] was averaged in the aforementioned non-codeine region, yielding an average intensity of $\overline{x} \pm 2\sigma = 18.0 \pm 8.2$ units. The m/e 298 ion intensity was 23.0 units and the m/e 298 ion thus belonged to the regional population.

The "blank" urine was compared to the Army spiked urines A, B, C, D (stored in glass bottles) in the non-codeine region by a simple pattern analysis. The peak intensities were normalized to sum to one:

$$\sum_{n=1}^{k} I_n = 1.0$$

[11]The 10 ng/ml A sample of morphine in Army donor urine stored in a polypropylene bottle was used as a blank urine, since the polypropylene surface was found to adsorb such levels of morphine.

Spectra were compared by calculating a discrepancy factor d:

$$d = \sum_{n=1}^{k} \left| I_n \text{ (spectrum x)} - I_n \text{ (spectrum y)} \right|$$

Values of d = 0 and 2.0 correspond to identical and totally dissimilar spectra respectively. Normal scatter in mass fragmentation patterns make a d value of ~ 0.2 an excellent match in comparing mass spectra of pure compounds versus library spectra. Comparison of a compound mass spectrum to that of structurally similar species generally yields d values of 0.7 to 1.6.[12] The susceptibility of CVA analysis to increased scatter in fragmentation patterns, due to high source pressure and thus enhanced gas phase and surface reactions, lead us to set a value of d ~ 0.4 as representing a good match.

Comparison of the "blank" urine to the Army urines A, B, C, D produced d factors of 0.23, 0.16, 0.46, 0.18 respectively. Thus the urines constitute good mass spectral matches in the region of interest.

Since the m/e 298 ion intensity is assumed to belong to the regional population for the case of a morphine-free urine, then if the m/e 298 ion intensity exceeds the value of $\overline{x} + 2\sigma$ calculated in the m/e 296-7, 301-7 region, a morphine-positive urine is indicated. One can subtract the value of $\overline{x}$ from x_{298} to obtain the ion intensity at m/e 298 due to codeine. This value, called $x_{codeine}$, is then ratioed

[12] L. R. Crawford and J. D. Morrison, Anal. Chem. 40, 1464 (1968).

to $\overline{x}$ to correct for changes in spectrometer sensitivity. The data indicates positive results for all the spiked urines and a negative for a blank (Table I). Thus, CVA detected down to 10 nanograms/milliliter morphine in urine. The CVA computer system should be set up to compute $\overline{x} + 2\sigma$, $x_{298} - (\overline{x} + 2\sigma)$, printout whether the sample is positive or negative for morphine, compute $(x_{298} - \overline{x})/\overline{x}$ and print out its value.

In addition, one could have a printout of a bar graph of $\overline{x}$ and $x_{298} - \overline{x}$. A simulation of such a graph, based on the data, is presented in Fig. 5.

A calibration curve of the data (concentration of morphine in urine versus $(x_{298} - \overline{x})/\overline{x}$ is non-linear in the high level region (Fig. 6). It is possible that at high morphine levels, the Methelute™ level utilized was insufficient to effect complete derivatization.

B. Army Urines -- Polypropylene Bottles.

The data was treated as in Section A. Results are presented in Table II and compared to Section A data in Table IV. Positives were scored for levels of 30 ng/ml morphine and above. The data indicates that polypropylene bottles adsorb morphine in urine to a greater extent (factor of ~x5) than untreated glass bottles (see Table IV).

C. Varian Donor Urines -- Glass Bottles.

Whereas the Army urines had been stored refrigerated, in the dark for two weeks, the Varian "glass bottle" urines were stored at room

temperature, room light conditions for one month.

These samples were scanned in the m/e 295-301 region. Whereas previous results (Sections A, B) showed m/e 298 to be the major codeine ion, in these samples, m/e 299 was the major ion observed. The shift from $(M-1)^+$ to $M\cdot^+$ ion dominance must be related to a change in source conditions. The data were treated as in Sections A, B although the limited scan gave only four ions from which to calculate $\overline{x}$(m/e 295, 296, 297, 301). The results are presented in Table III and are compared to the previous data in Table IV. The results indicate that room temperature, room light conditions cause significant sample loss (factor of ~ x5 to x10) relative to refrigerated, dark storage.

SUMMARY AND CONCLUSIONS

CVA sensitivity to morphine in urine at 10 ng/ml was demonstrated, utilizing a derivatization technique. Data treatment involved computation of an average urinary background in the m/e region of the major morphine derivative ion and comparison of that ion intensity versus the background.

The demonstrated sensitivity of 10 ng/ml will allow:

1) Morphine detection of un-hydrolyzed urines collected within 24 hours of dose.

2) Morphine detection in hydrolyzed urines greater than 3-4 days after dose. The exact post-dose time limits on detection are unknown as data on urinary morphines beyond three days post-dose is not available.

Urine samples can be stored refrigerated, at least two weeks, in untreated glass bottles without significant sample loss. Storage in polypropylene bottles and storage under room temperature, room light conditions cause significant sample loss.

MORPHINE ANALYSIS -- CVA-METHYLATION

TABLE I. Army urines, stored two weeks, refrigerated in the dark, in glass bottles.

SAMPLE	$\bar{x} \pm 2\sigma$	$\bar{x} + 2\sigma$	x_{298}	$x_{298}-(\bar{x}+2\sigma)$	$\pm$	$(x_{298}-\bar{x})/\bar{x}$
Blank	18.0±8.2	26.2	23.0	- 3.2	-	0.28
A 10 ng/ml	7.8±3.1	10.9	18.0	+ 7.1	+	1.44
B 30 ng/ml	9.9±5.9	15.8	45.0	+ 29.2	+	3.54
C 100 ng/ml	18.5±26.0	44.5	110.0	+ 65.5	+	4.95
D 300 ng/ml	9.5±3.8	13.3	150.0	+136.7	+	14.8

MORPHINE ANALYSIS -- CVA-METHYLATION

TABLE II. Army urines, stored two weeks, refrigerated in the dark, in polypropylene bottles.

SAMPLE	$\overline{x} \pm 2\sigma$	$\overline{x} + 2\sigma$	x_{298}	$x_{298}-(\overline{x}+2\sigma)$	$\pm$	$(x_{298}-\overline{x})/\overline{x}$
Blank	18.0±8.2	26.2	23.0	- 3.2	-	0.28
A 10 ng/ml	18.0±9.2	27.2	23.0	- 4.2	-	0.28
B 30 ng/ml	41.5±13.3	54.8	80.0	+25.2	+	0.95
C 100 ng/ml	47.9±15.0	62.9	150.0	+87.1	+	2.14
D 300 ng/ml	4.9±3.1	8.0	14.0	+ 6.0	+	1.85
E 1 µg/ml	53.7±14.5	68.2	150.0	+81.8	+	1.80

MORPHINE ANALYSIS -- CVA-METHYLATION

TABLE III. Varian urines, stored one month, room temperature, room light conditions, glass bottles.

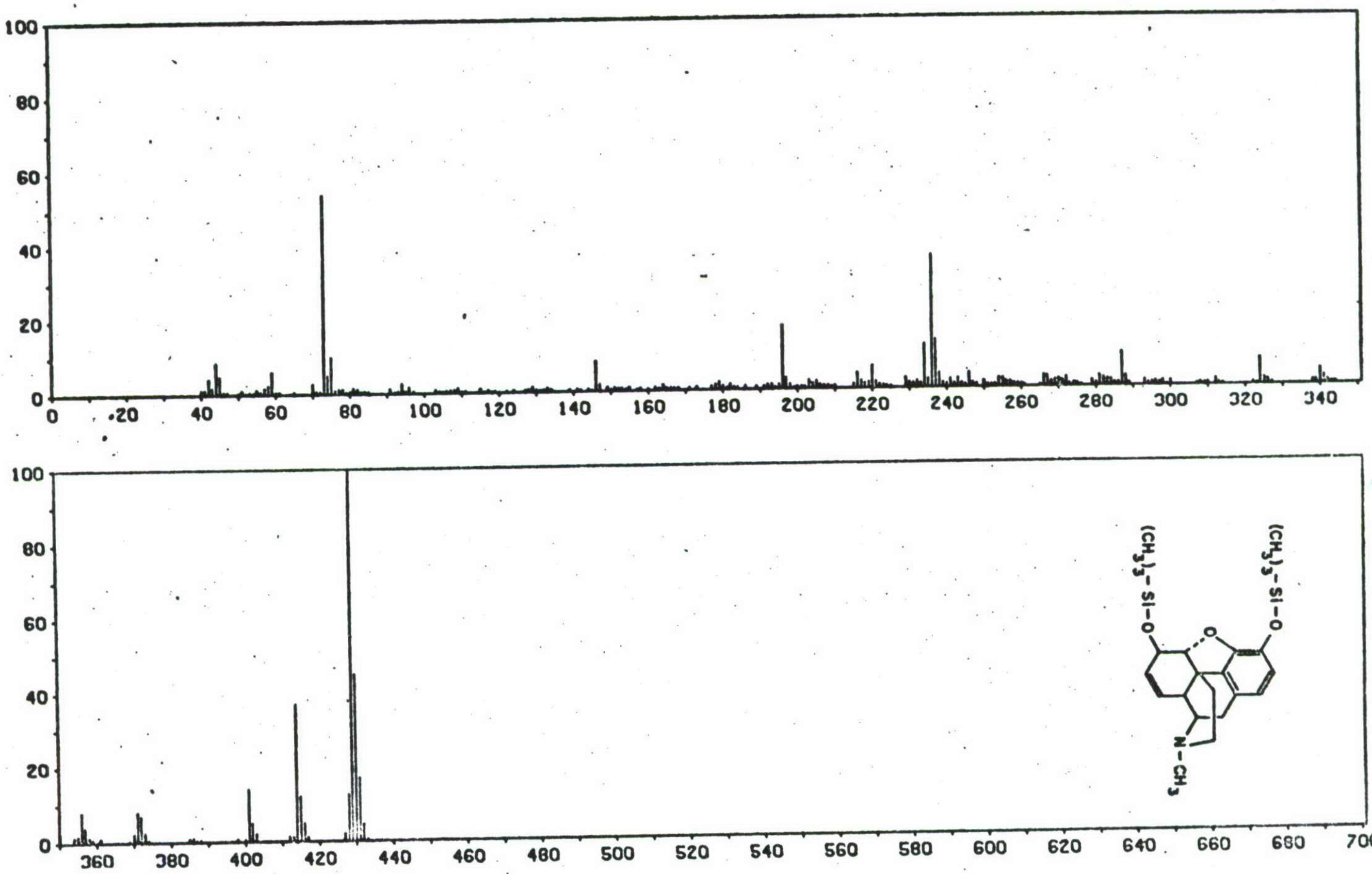
SAMPLE	$\bar{x} \pm 2\sigma$	$\bar{x} + 2\sigma$	x_{299}	$x_{299}-(\bar{x}+2\sigma)$	±	$x_{299}-\bar{x}/\bar{x}$
Varian Blank	20±7.6	27.6	26	- 1.6	-	0.30
100 ng/ml	13.3±4.4	17.7	27	+ 9.3	+	+1.03
500 ng/ml	31.5±5.0	36.5	56	+19.5	+	+0.80
1 μg/ml	40.3±6.6	46.9	90	+50.3	+	+1.25

TABLE IV. Comparison of storage conditions versus codeine signals--CVA-Methylation analysis of Army, Varian urines for morphine.

$$\frac{x_{298} - \bar{x}}{\bar{x}}$$

	100 ng/ml	300 ng/ml
I. Army urines		
--glass bottles	+4.95	+14.8
--polypropylene	+2.14	+ 1.85
II. Varian urines		
--glass bottles room temperature room light	+1.03	+ 0.80 (for 500 ng/ml)

FIGURE 2. MASS SPECTRUM OF BIS (O-TRIMETHYLSILYL) MORPHINE



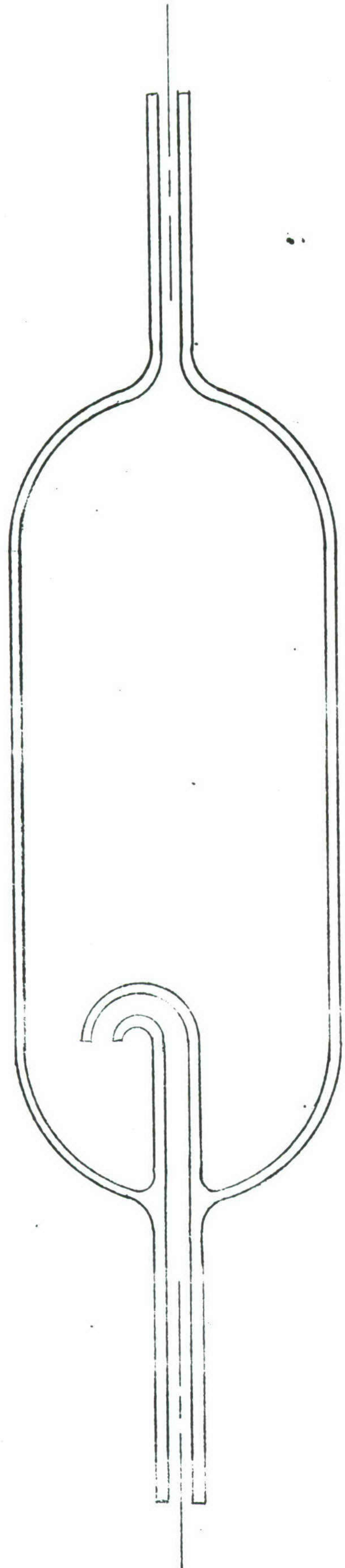
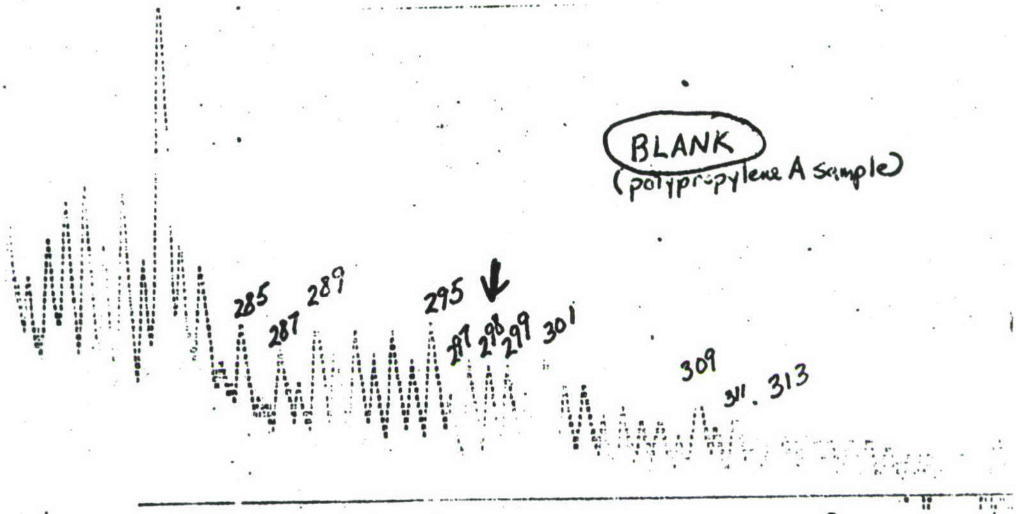
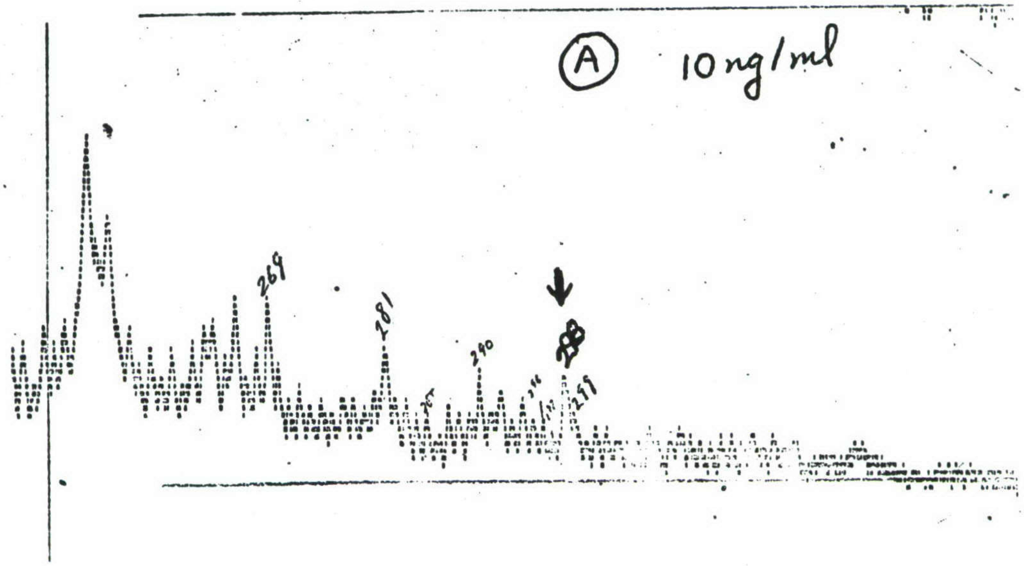


FIG. 3 GLASS INLET AND EXPANSION CHAMBER

FIGURE 4A-D. MASS SPECTRA OF METHYLATED URINE EXTRACTS, BLANK AND SAMPLES A THROUGH D, STORAGE IN UNTREATED GLASS BOTTLES, ARMY DONOR.





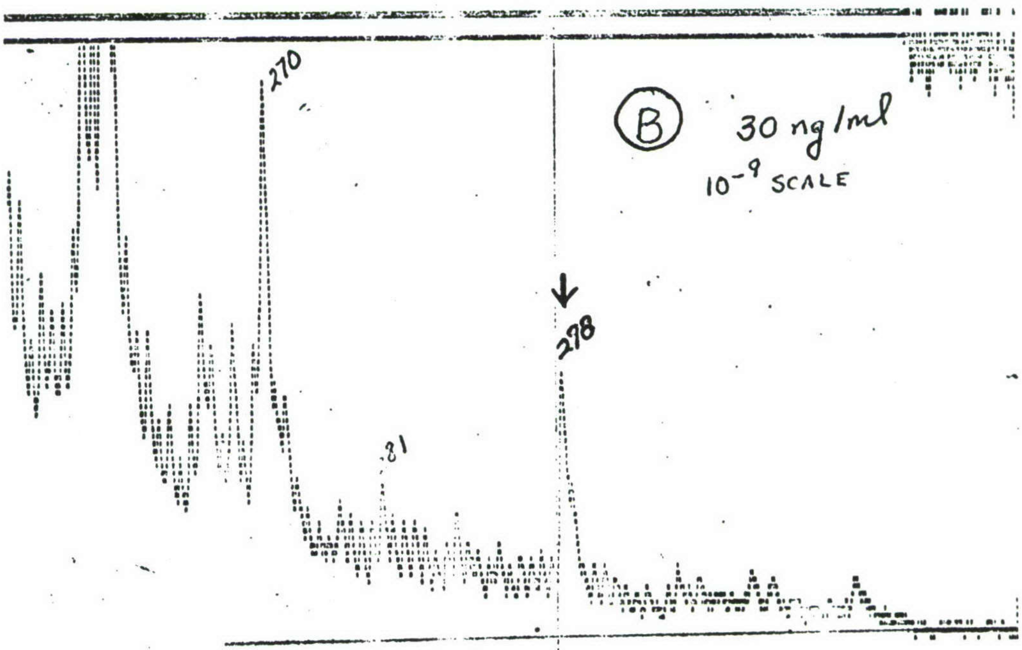
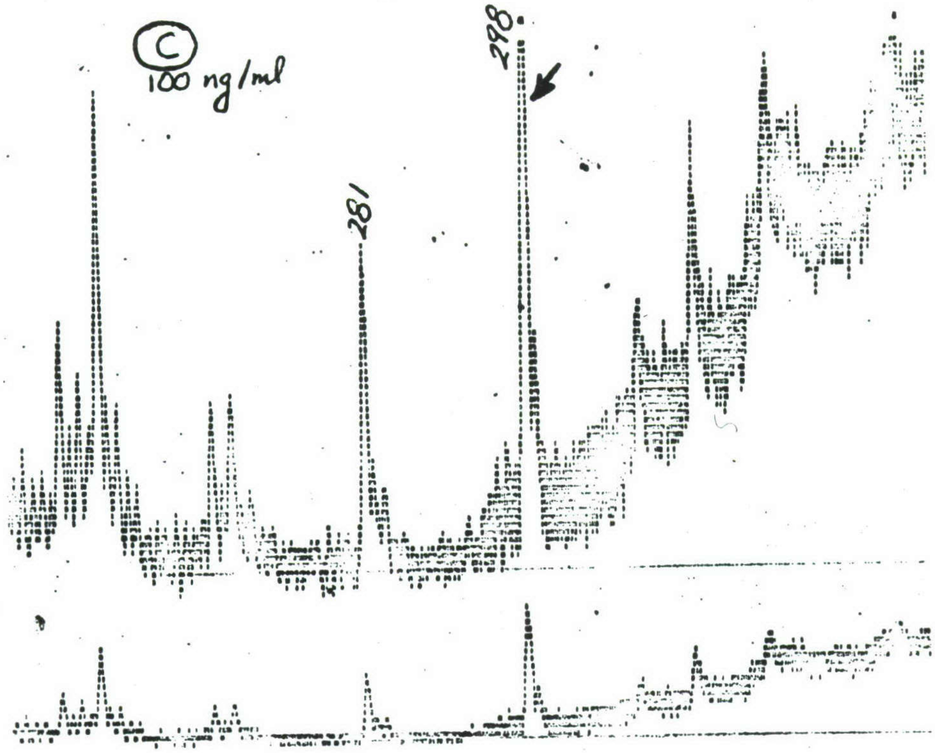


FIGURE 4A-D (CONTINUED)



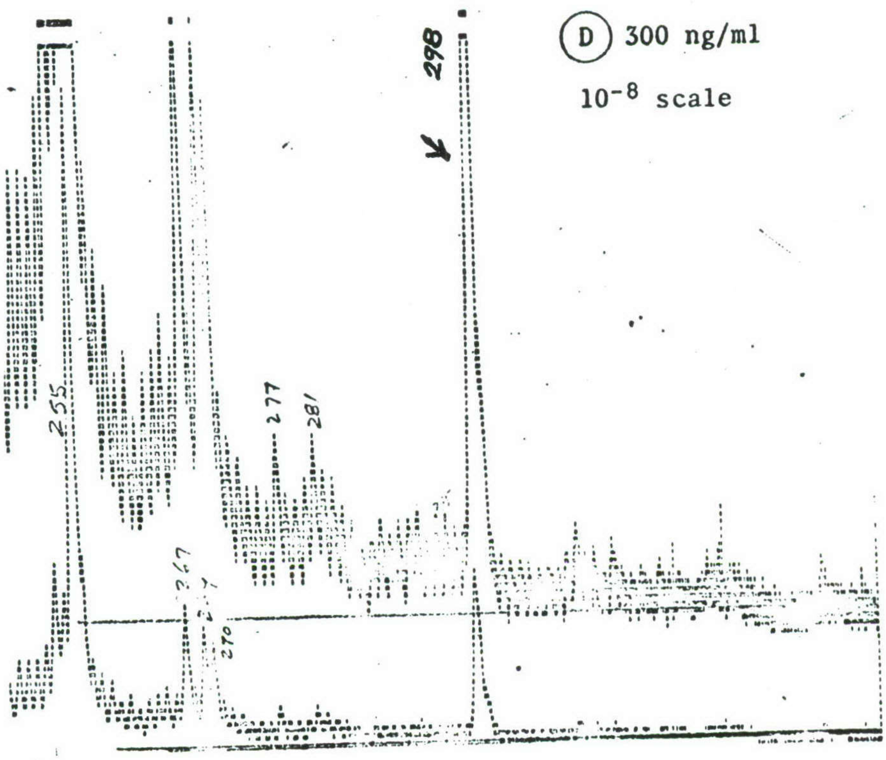


FIGURE 5. $\overline{x}$, $(x_{298} - \overline{x})$ PLOTS

ARMY URINES, STORAGE IN GLASS BOTTLES

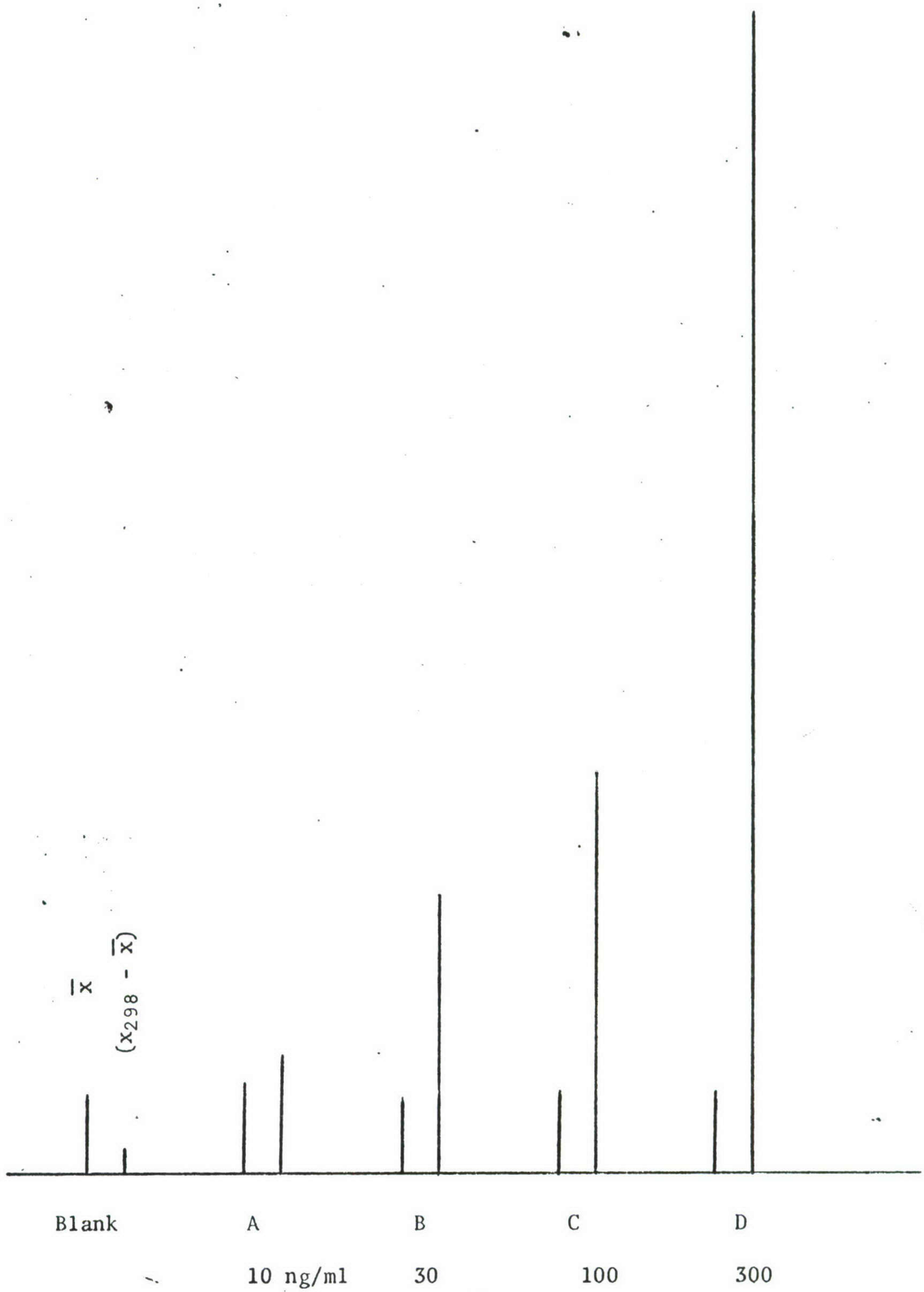
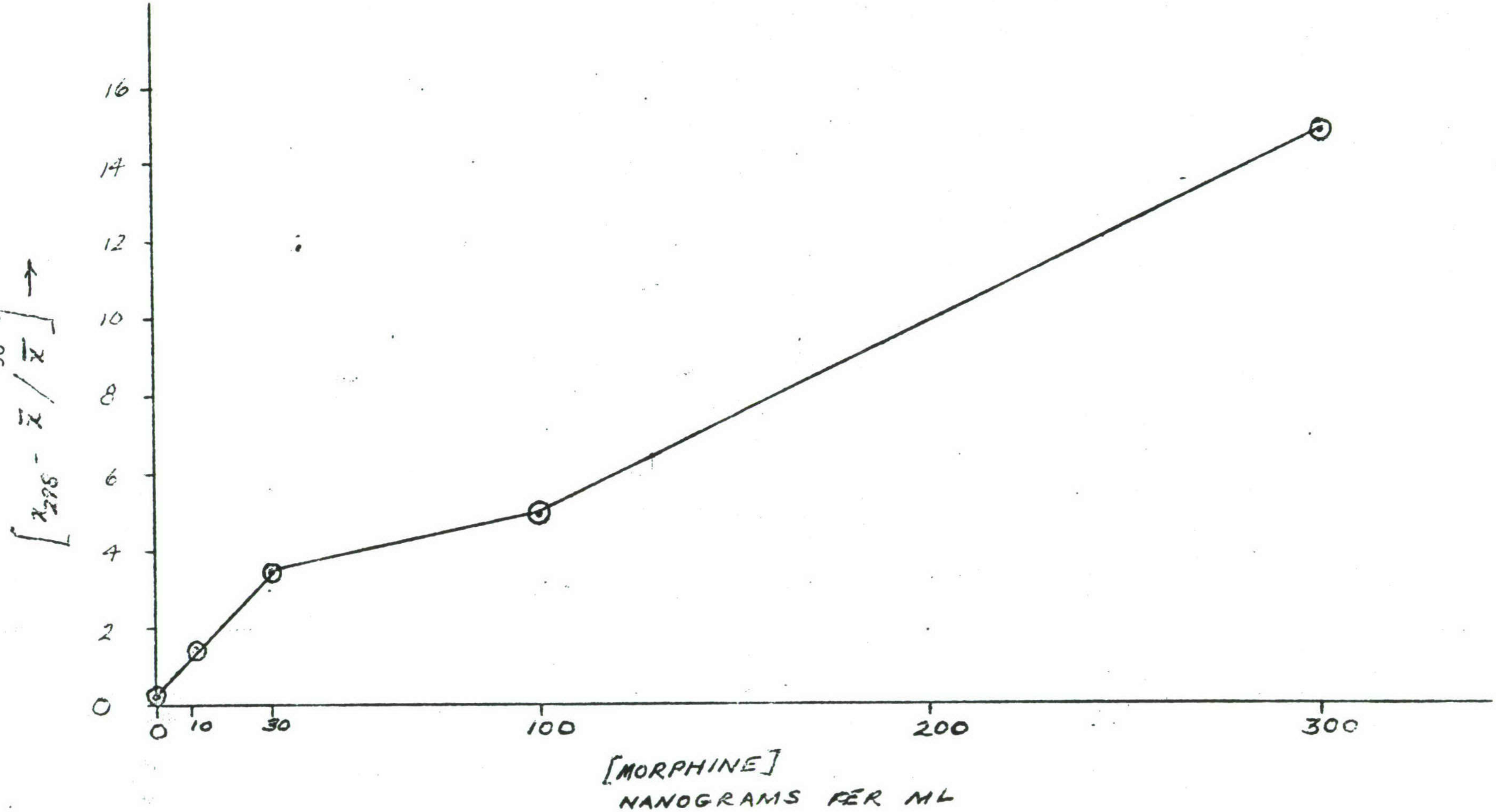
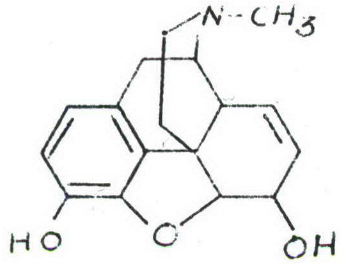


Figure 6. CALIBRATION CURVE, MORPHINE IN URINE - ARMY URINES, STORAGE IN UNTREATED GLASS BOTTLES.

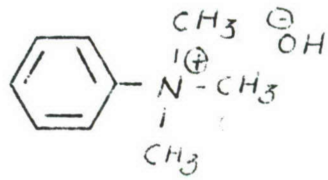


APPENDICES

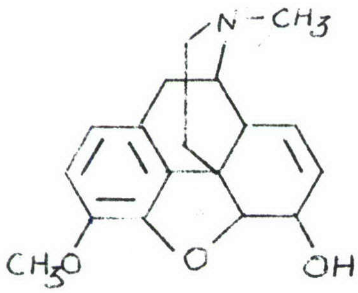
APPENDIX I. METHYLATION OF MORPHINE



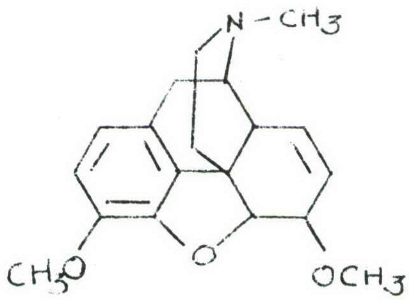
Morphine
MW 285
m.p. 230°C



Trimethylanilinium hydroxide (TMAH)



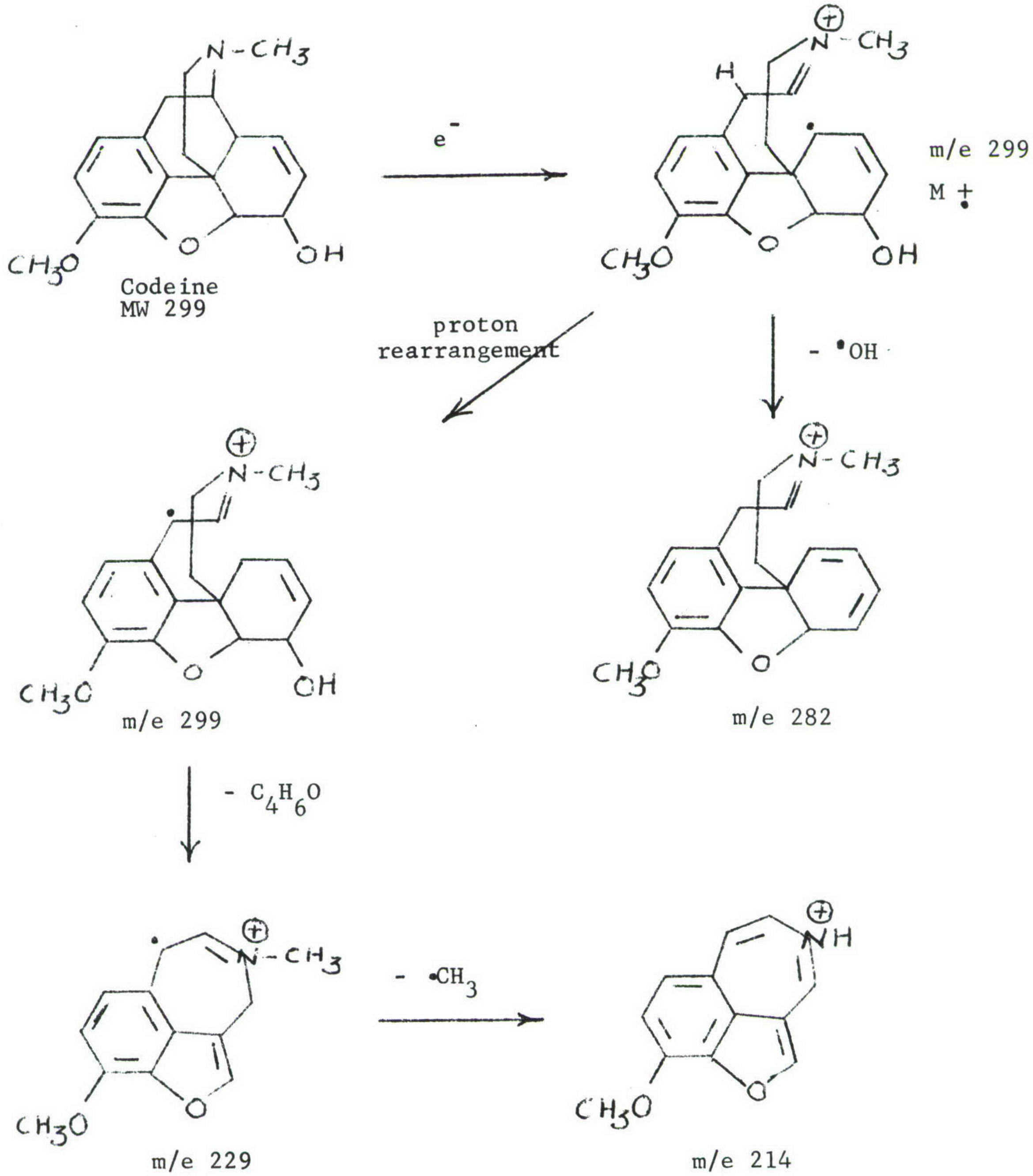
Codeine
(Monomethylmorphine)
MW 299 mp 157°C



Dimethylmorphine
MW 313 mp below 157°C

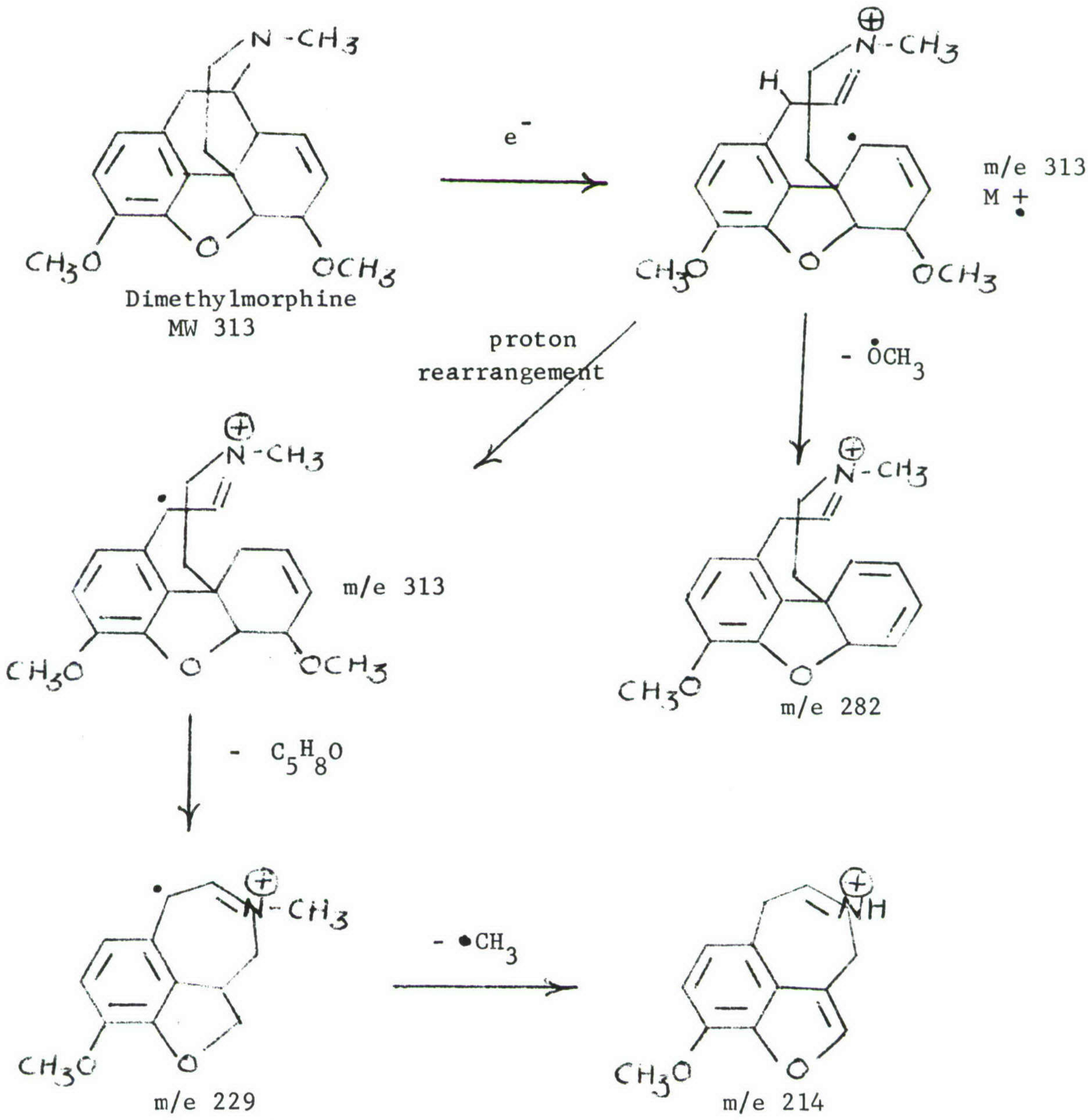
TMAH reacts instantaneously (on the order of seconds) with morphine to form codeine and a minor amount of dimethylmorphine.

APPENDIX II. MASS FRAGMENTATION SCHEME OF CODEINE (MONOMETHYLMORPHINE)



In addition to the strong molecular ions at m/e 299 and m/e 313 in the mass spectra of mono and dimethylmorphine, both compounds produce strong fragment ions at m/e 282, 268 and 229. See following page for fragmentation scheme of dimethylmorphine.

APPENDIX III. MASS FRAGMENTATION SCHEME OF DIMETHYLMORPHINE



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Walter Reed Institute of Research
Division of Biochemistry
Washington, DC 20012

Dr. Charles E. Becker 1
San Francisco General Hospital
22d & Potrero Avenue
San Francisco, CA 94101

Dr. Robert S. Melville 1
Research Grants Branch
National Institute of General Medical Science
Westwood Building, Room 938
5333 Westford Avenue
Bethesda, MD 20016

Dr. Henry Fales 1
Chief, Laboratory of Chemistry
National Heart and Lung Institute
Building 10 7N306
9000 Rockville Pike
Bethesda, MD 20016

Dr. George J. Cosmides/Dr. Bryon B. Clark 1
Program Coordinator Pharmacology-Toxicology
National Institute of General Medical Science
Westwood Building, Room 9A03
5333 Westford Avenue
Bethesda, MD 20016

Mr. Robert Mills 1
Law Enforcement Standards Laboratory
National Bureau of Standards
Building 221
Washington, DC 20234

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Department of Transportation
Technical Information Branch
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Chief, Laboratory of Chemical Pharmacology
National Cancer Institute
Building 10, Room 6N119
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Bethesda, MD 20014

Dr. Eric Nelson 1
Nelson Research
19722 Jamboree Blvd.
Irvine, CA 92664

Dr. Thomas Davis 1
Smith Kline Instruments
440 Page Mill Road
Palo Alto, CA 94303

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Santa Clara County Laboratory of Criminalistics
875 N. San Pedro
San Jose, CA 95100

Mr. Walter P. Hay 1
US Department of Treasury
IRS Alcohol, Tobacco & Firearms Division
1111 Constitution Avenue
Washington, DC 20224

Dr. R. A. Rasmussen 1
Department of Biochemistry
Washington State University
Pullman, WA 99163

Professor Robert Beaudet 1
Department of Chemistry
UCLA
Los Angeles, CA 90024

Professor John Baldeschwieler 1
Department of Chemistry
California Institute of Technology
Pasadena, CA 91100

Mr. Joseph Koles 1
Drug Enforcement Agency
Special Testing and Research Laboratory
Washington, DC 20537

Mr. Leo DalCortivo 1
Chief Toxicologist
Suffolk County Medical Examiners Office
Hauppage, L.I., NY 11787

Mr. Lester D. Shubin 1
Law Enforcement Assistance Administration
633 Indiana Avenue
Washington, DC 20530

Mr. William Butler 1
Drug Enforcement Agency
Washington, DC 20537

Mr. Charles Gaskin 1
Drug Enforcement Agency
Washington, DC 20537

Dr. A. E. Barrington 1
DOT/Transportation System Center
55 Broadway
Cambridge, MA 02142

Dr. John R. Hobbs 1
Biotechnology Division TIM
DOT/Transportation Systems Center
55 Broadway
Cambridge, MA 02142

Commander 1
Edgewood Arsenal
ATTN: Dr. Robert I. Ellin
Chemical Research Laboratory
Aberdeen Proving Ground, MD 21010

Dr. Bernard Newman 1
Suffolk County Police Department
Hauppage, L. I., NY 11787

Dr. Charles W. Gorodetzky 1
US Department of Health, Education and Welfare
NIMH Addiction Research Center
P.O. Box 2000
Lexington, KY 40507